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(54) Title: HUMAN PROTEINS HAVING HYDROPHOBIC DOMAINS AND DNAS ENCODING THESE PROTEINS

### (57) Abstract

The present invention provides human proteins having hydrophobic domains, DNAs coding for these proteins, and expression vectors for these DNAs as well as eucaryotic cells expressing these DNAs.

3

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#### DESCRIPTION

# Human Proteins Having Hydrophobic Domains and DNAs Encoding These Proteins

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#### TECHNICAL FIELD

The present invention relates to human proteins having hydrophobic domains, DNAs coding for these proteins, and expression vectors for these DNAs as well as eucaryotic cells expressing these DNAs. The proteins of the present invention can be employed as pharmaceuticals or as antigens for preparing antibodies against these proteins. The human cDNAs of the present invention can be utilized as probes for the genetic diagnosis and gene sources for the gene therapy. Furthermore, the cDNAs can be utilized as gene sources for large-scale production of the proteins encoded by these cDNAs. Cells into which these genes are introduced to express secretory proteins and membrane proteins in large amounts can be utilized for detection of the corresponding receptors and ligands, screening of novel low-molecular pharmaceuticals, and so on.

#### BACKGROUND ART

Cells secrete many proteins outside the cells. These secretory proteins play important roles for the proliferation control, the differentiation induction, the material transportation, the biological protection, etc. in the cells. Different from intracellular proteins, the secretory proteins exert their actions outside the cells, whereby they can be administered in the intracorporeal manner such as the injection or the drip, so that there are

2

hidden potentialities as medicines. In fact, a number of human secretory proteins such as interferons, interleukins, thrombolytic agents, etc. have erythropoietin, In addition, currently employed as medicines. secretory proteins other than those described above have undergoing clinical trials to develop as pharmaceuticals. Because it has been conceived that the human cells still produce many unknown secretory proteins, availability of these secretory proteins as well as genes coding for them is expected to lead to development of novel pharmaceuticals utilizing these proteins.

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On the other hand, membrane proteins play important roles, as signal receptors, ion channels, transporters, etc. material transportation and the transmission through the cell membrane. Examples thereof include receptors for a variety of cytokines, ion channels for the sodium ion, the potassium ion, the chloride ion, etc., transporters for saccharides and amino acids, and so on, where the genes for many of them have been cloned already. It has been clarified that abnormalities of these membrane proteins are associated with a number of hithertocryptogenic diseases. Therefore, discovery of a new membrane protein is anticipated to lead to elucidation of the causes of many diseases, so that isolation of a new gene coding for the membrane protein has been desired.

Heretofore, owing to difficulty in the purification from human cells, these secretory proteins and membrane proteins have been isolated by an approach from the gene side. A general method is the so-called expression cloning which comprises introduction of a cDNA library into eucaryotic cells to express cDNAs and then screening of the cells secreting, or expressing on the surface of membrane,

3

the objective active protein. However, this method is applicable only to cloning of a gene for a protein with a known function.

In general, secretory proteins and membrane proteins possess at least one hydrophobic domain inside the proteins, wherein, after synthesis thereof in the ribosome, this domain works as a secretory signal or remains in the phospholipid membrane to be trapped in the membrane. Accordingly, the evidence of this cDNA for encoding a secretory protein and a membrane protein is provided by determination of the whole base sequence of a full-length cDNA followed by detection of highly hydrophobic domain(s) in the amino acid sequence of the protein encoded by this cDNA.

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# OBJECTS OF THE INVENTION

The main object of the present invention is to provide novel human proteins having hydrophobic domains, DNAs coding for these proteins, and expression vectors for these DNAs as well as transformed eucaryotic cells that are capable of expressing these DNAs. This object as well as other objects and advantages of the present invention will become apparent to those skilled in the art from the following description with reference to the accompanying drawings.

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# BRIEF DESCRIPTION OF DRAWINGS

- Fig. 1 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01550.
- Fig. 2 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02593.
  - Fig. 3 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10195.

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- Fig. 4 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10423.
- Fig. 5 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10506.
- Fig. 6 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10507.

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- Fig. 7 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10548.
- Fig. 8 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10566.
- Fig. 9 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10567.
- Fig. 10 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10568.
- Fig. 11 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01426.
- Fig. 12 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02515.
- Fig. 13 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02575.
- Fig. 14 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10357.
- Fig. 15 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10447.
- Fig. 16 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10477.
  - Fig. 17 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10513.
- Fig. 18 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10540.
- Fig. 19 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10557.

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Fig. 20 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10563.

Fig. 21 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01467.

Fig. 22 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01956.

Fig. 23 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02545.

Fig. 24 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02551.

Fig. 25 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02631.

Fig. 26 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02632.

Fig. 27 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10488.

Fig. 28 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10538.

Fig. 29 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10542.

Fig. 30 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10571.

Fig. 31 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01470.

Fig. 32 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02419.

Fig. 33 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02631.

Fig. 34 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02695.

Fig. 35 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10031.

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- Fig. 36 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10530.
- Fig. 37 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10541.
- Fig. 38 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10550.
- Fig. 39 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10590.
- Fig. 40 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10591.
- Fig. 41 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP01462.
- Fig. 42 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02485.
- Fig. 43 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP02798.
- Fig. 44 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10041.
- Fig. 45 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10246.
- Fig. 46 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10392.
- Fig. 47 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10489.
- Fig. 48 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10519.
- Fig. 49 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10531.
- Fig. 50 illustrates the hydrophobicity/hydrophilicity profile of the protein encoded by clone HP10574.

PCT/JP99/03929

the result of intensive studies, the present inventors have been successful in cloning of cDNAs coding for proteins having hydrophobic domains from the human fulllength cDNA bank, thereby completing the present invention. words, the present invention provides hydrophobic domains, proteins having namely proteins comprising any of the amino acid sequences represented by SEQ ID Nos. 1 to 10, 31 to 40, 61 to 70, 91 to 100, and 121 to 130. Moreover, the present invention provides DNAs coding for the above-mentioned proteins, exemplified by cDNAs comprising any of the base sequences represented by SEQ ID Nos. 11 to 20, 41 to 50, 71 to 80, 101 to 110, and 131 to 140, as well as expression vectors that are capable of expressing any of these DNAs by in vitro translation or in eucaryotic cells and transformed eucaryotic cells that are capable of expressing these DNAs and of producing the abovementioned proteins.

#### DETAILED DESCRIPTION OF THE INVENTION

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The proteins of the present invention can be obtained, for example, by a method for isolation from human organs, cell lines, etc., a method for preparation of peptides by the chemical synthesis, or a method for production with the recombinant DNA technology using the DNAs coding for the hydrophobic domains of the present invention, among which the method for production with the recombinant DNA technology is employed preferably. For instance, in vitro expression of the proteins can be achieved by preparation of an RNA by in vitro transcription from a vector having one of the cDNAs of the present invention, followed by in vitro translation using this RNA as a template. Also, introduction of the translated region into a suitable expression vector

8

by the method known in the art leads to expression of a large amount of the encoded protein in prokaryotic cells such as *Escherichia coli*, *Bacillus subtilis*, etc., and eucaryotic cells such as yeasts, insect cells, mammalian cells, etc.

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In the case where one of the proteins of the present invention is produced by expressing the DNA by in vitro translation, the protein of the present invention can be produced in vitro, when the translated region of this cDNA introduced into a vector having an RNA polymerase promoter, followed by addition of the vector to an in vitro translation system such as a rabbit reticulocyte lysate or a extract, containing an RNA polymerase corresponding to the promoter. RNA polymerase promoters are exemplified by T7, T3, SP6, and the like. The vectors containing these RNA polymerase promoters are exemplified by pKA1, pCDM8, pT3/T7 18, pT7/3 19, pBluescript II, and so on. Furthermore, the protein of the present invention can be expressed as the secreted form or the form incorporated into the microsome membrane, when a canine pancreas microsome or the like is added to the reaction system.

In the case where one of the protein of the present expressing the invention is produced by DNA microorganism such as Escherichia coli etc., a recombinant expression vector bearing the translated region of the cDNA of the present invention is constructed in an expression vector having an origin which can be replicated in the microorganism, a promoter, a ribosome-binding site, a cDNAcloning site, a terminator etc. and, after transformation of the host cells with this expression vector, the resulting transformant is incubated, whereby the protein encoded by said cDNA can be produced on a large scale

9

microorganism. In this case, a protein fragment containing any region can be obtained by carrying out the expression with inserting an initiation codon and a termination codon in front of and behind the selected translated region. Alternatively, a fusion protein with another protein can be expressed. Only the portion of the protein encoded by this cDNA can be obtained by cleavage of this fusion protein with a suitable protease. The expression vector for Escherichia coli is exemplified by the pUC series, pBluescript II, the pET expression system, the pGEX expression system, and so on.

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In the case where one of the proteins of the present invention is produced by expressing the DNA in eucaryotic cells, the protein of the present invention can be produced as a secretory protein or as a membrane protein on the cellmembrane surface, when the translated region of this cDNA is introduced into an expression vector for eucaryotic cells that has a promoter, a splicing region, a poly(A) addition site, etc., followed by introduction into the eucaryotic The expression vector is exemplified by pKA1, pED6dpc2, pCDM8, pSVK3, pMSG, pSVL, pBK-CMV, pBK-RSV, EBV vector, pRS, pYES2, and so on. Examples of eucaryotic cells to be used in general include mammalian cultured cells such as simian kidney cells COS7, Chinese hamster ovary cells CHO, etc., budding yeasts, fission yeasts, silkworm cells, Xenopus oocytes, and so on, but any eucaryotic cells may be used, provided that they are capable of expressing the proteins of the present invention. The expression vector can be introduced into the eucaryotic cells by methods known in the art such as the electroporation method, the calcium phosphate method, the liposome method, the DEAE-dextran method, and so on.

After one of the proteins of the present invention is

WO 00/05367

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PCT/JP99/03929

expressed in prokaryotic cells or eucaryotic cells, the objective protein can be isolated from the culture and purified by a combination of separation procedures known in the art. Such examples include treatment with a denaturing agent such as urea or a detergent, sonication, enzymatic digestion, salting-out or solvent precipitation, dialysis, centrifugation, ultrafiltration, gel filtration, SDS-PAGE, isoelectric focusing, ion-exchange chromatography, hydrophobic chromatography, affinity chromatography, reverse phase chromatography, and so on.

The proteins of the present invention include peptide fragments (5 amino acid residues or more) containing any partial amino acid sequence in the amino acid sequences represented by SEQ ID Nos. 1. to 10, 31 to 40, 61 to 70, 91 to 100, and 121 to 130. These peptide fragments can be utilized as antigens for preparation of antibodies. Hereupon, among the proteins of the present invention, those having the signal sequences are secreted in the form of mature proteins, after the signal sequences are removed. Therefore, these mature proteins shall come within the scope of the present invention. The N-terminal amino acid sequences of the mature proteins can be easily determined by using the method for the determination of cleavage site of a signal Furthermore, sequence ſJP 8-187100 A]. some proteins undergo the processing on the cell surface to be converted to the secretory forms. Such proteins or peptides in the secretory forms shall come within the scope of the present invention. In the case where sugar chain-binding sites are present in the amino acid sequences, expression in appropriate eucaryotic cells affords proteins to which sugar chains are attached. Accordingly, such proteins or peptides to which sugar chains are attached shall come within the

scope of the present invention.

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The DNAs of the present invention include all the DNAs coding for the above-mentioned proteins. These DNAs can be obtained by using a method by chemical synthesis, a method by cDNA cloning, and so on.

The cDNAs of the present invention can be cloned, for example, from cDNA libraries derived from the human cells. These cDNAs are synthesized by using as templates poly(A)\* RNAs extracted from human cells. The human cells may be cells delivered from the human body, for example, by the operation or may be the cultured cells. The cDNAs can be synthesized by using any method selected from the Okayama-Berg method [Okayama, H. and Berg, P., Mol. Cell. Biol. 2: 161-170 (1982)], the Gubler-Hoffman method [Gubler, U. and Hoffman, J. Gene 25: 263-269 (1983)], and so on, but it is preferred to use the capping method [Kato, S. et al., Gene 150: 243-250 (1994)], as exemplified in Examples, in order to obtain a full-length clone in an effective manner. In addition, commercially available, human cDNA libraries can be utilized. Cloning of the cDNAs of the present invention from the cDNA libraries can be carried out by synthesis of an oligonucleotide on the basis of base sequences of any portion in the cDNA of the present invention, followed by screening using this oligonucleotide as the probe according to the colony or plaque hybridization by a method known in the art. In addition, the cDNA fragments of the present invention can be prepared by synthesis of oligonucleotides which hybridize with both termini of the objective cDNA fragment, followed by the usage of these oligonucleotides as the primers for the RT-PCR method using an mRNA isolated from human cells.

The cDNAs of the present invention are characterized by

12

comprising either of the base sequences represented by SEQ ID Nos. 11 to 20, 41 to 50, 71 to 80, 101 to 110, and 131 to 140 or the base sequences represented by SEQ ID Nos. 21 to 30, 51 to 60, 81 to 90, 111 to 120, and 141 to 150. Table 1 summarizes the clone number (HP number), the cells from which the cDNA was obtained, the total base number of the cDNA, and the number of the amino acid residues of the encoded protein, for each of the cDNAs.

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Table 1					
SEQ ID No.	HP number	Cells	Base number	Number of amino acid residues	
1, 11, 21	HP01550	Stomach cancer	510	125	
2, 12, 22	HP02593	Saos-2	697	131	
3, 13, 23	HP10195	HT-1080	1619	242	
4, 14, 24	HP10423	U-2 OS	1066	264	
5, 15, 25	HP10506	Stomach cancer	618	112	
6, 16, 26	HP10507	Stomach cancer	1021	146	
7, 17, 27	HP10548	Stomach cancer	1432	344	
8, 18, 28	HP10566	Stomach cancer	601	97	
9, 19, 29	HP10567	Stomach cancer	585	124	
10, 20, 30	HP10568	Stomach cancer	1100	327	
31, 41, 51	HP01426	Stomach cancer	1065	313	
32, 42, 52	HP02515	Saos-2	937	229	
33, 43, 53	HP02575	Saos-2	1678	467	
34, 44, 54	HP10357	Stomach cancer	467	99	
35, 45, 55	HP10447	Liver	875	189	
36, 46, 56	HP10477	Liver	1256	363	
37, 47, 57	HP10513	Stomach cancer	884	249	
38, 48, 58	HP10540	Saos-2	589	98	
39, 49, 59	HP10557	Stomach cancer	673	172	
40, 50, 60	HP10563	Saos-2	1425	120	
61, 71, 81	HP01467	HT-1080	1436	307	
62, 72, 82	HP01956	Liver	997	183	
63, 73, 83	HP02545	Saos-2	1753	327	
64, 74, 84	HP02551	Saos-2	1117	223	
65, 75, 85	HP02631	Saos-2	1380	48	
66, 76, 86	HP02632	HT-1080	1503	371	
67, 77, 87	HP10488	Liver	733	90	
68, 78, 88	HP10538	Saos-2	3768	499	
69, 79, 89	HP10542	Stomach cancer	770	106	
70, 80, 90	HP10571	Stomach cancer	1229	152	

WO 00/05367 PCT/JP99/03929

91, 101, 111	HP01470	Stomach cancer	1619	358	
92, 102, 112	HP02419	Stomach cancer	2054	226	
93, 103, 113	HP02631	Saos-2	1380	195	
94, 104, 114	HP02695	Stomach cancer	1292	339	
95, 105, 115	HP10031	Saos-2	2168	487	
96, 106, 116	HP10530	Saos-2	1357	393	
97, 107, 117	HP10541	Stomach cancer	711	196	
98, 108, 118	HP10550	Stomach cancer	651	107	
99, 109, 119	HP10590	HT-1080	1310	350	
100, 110, 120	HP10591	HT-1080	1400	107	
121, 131, 141	HP01462	HT-1080	2050	483	
122, 132, 142	HP02485	Stomach cancer	2746	334	
123, 133, 143	HP02798	HT-1080	1136	267	
124, 134, 144	HP10041	Saos-2	619	106	
125, 135, 145	HP10246	кв	864	224	
126, 136, 146	HP10392	U-2 OS	1527	258	
127, 137, 147	HP10489	Stomach cancer	659	110	
128, 138, 148	HP10519	Stomach cancer	710	91	
129, 139, 149	HP10531	Saos-2	2182	344	
130, 140, 150	HP10574	Stomach cancer	2773	428	

Hereupon, the same clones as the cDNAs of the present invention can be easily obtained by screening of the cDNA libraries constructed from the human cell lines or human tissues utilized in the present invention by the use of an oligonucleotide probe synthesized on the basis of the cDNA base sequence described in any of SEQ ID Nos. 11 to 30, 41 to 60, 71 to 90, 101 to 120, and 131 to 150.

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In general, the polymorphism due to the individual difference is frequently observed in human genes. Accordingly, any cDNA in which one or plural nucleotides are inserted, deleted and/or substituted with other nucleotides in SEQ ID Nos. 11 to 30, 41 to 60, 71 to 90, 101 to 120, and

131 to 150 shall come within the scope of the present invention.

In a similar manner, any protein in which one or plural amino acids are inserted, deleted and/or substituted with other amino acids shall come within the scope of the present invention, as far as the protein possesses the activity of any protein having the amino acid sequences represented by SEQ ID Nos. 1 to 10, 31 to 40, 61 to 70, 91 to 100, and 121 to 130.

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The cDNAs of the present invention include cDNA fragments (10 bp or more) containing any partial base sequence in the base sequences represented by SEQ ID Nos. 11 to 20, 41 to 50, 71 to 80, 101 to 110, and 131 to 140 or in the base sequences represented by SEQ ID Nos. 21 to 30, 51 to 60, 81 to 90, 111 to 120, and 141 to 150. Also, DNA fragments consisting of a sense strand and an anti-sense strand shall come within this scope. These DNA fragments can be utilized as the probes for the genetic diagnosis.

In addition to the activities and uses described above, the polynucleotides and proteins of the present invention may exhibit one or more of the uses or biological activities (including those associated with assays cited herein) identified below. Uses or activities described for proteins of the present invention may be provided by administration or use of such proteins or by administration or use of polynucleotides encoding such proteins (such as, for example, in gene therapies or vectors suitable for introduction of DNA).

# Research Uses and Utilities

The polynucleotides provided by the present invention can be used by the research community for various purposes. The polynucleotides can be used to express recombinant

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PCT/JP99/03929

protein for analysis, characterization or therapeutic use; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in disease states); as molecular weight markers on Southern gels; as chromosome markers or tags (when labeled) identify chromosomes or to map related gene positions; to DNA sequences compare with endogenous in patients to identify potential genetic disorders; as probes to hybridize and thus discover novel, related DNA sequences; as a source of information to derive PCR primers for fingerprinting; as a probe to "subtract-out" known sequences in the process of discovering other novel polynucleotides; for selecting and making oligomers for attachment to a "gene other support, including for examination of expression patterns; to raise anti-protein antibodiesusing DNA immunization techniques; and as an antigen to raise anti-DNA antibodies or elicit another immune response. Where the polynucleotide encodes a protein which binds or potentially binds to another protein (such as, for example, in a receptor-liqand interaction), the polynucleotide can also be used in interaction trap assays (such as, for example, that described in Gyuris et al., Cell 75:791-803 (1993)) to identify polynucleotides encoding the other protein with which binding occurs or to identify inhibitors of the binding interaction.

The proteins provided by the present invention can similarly be used in assay to determine biological activity, including in a panel of multiple proteins for high-throughput screening; to raise antibodies or to elicit another immune response; as a reagent (including the labeled reagent) in assays designed to quantitatively determine

PCT/JP99/03929

levels of the protein (or its receptor) in biological fluids; as markers for tissues in which the corresponding protein is preferentially expressed (either constitutively or at a particular stage of tissue differentiation or development or in a disease state); and, of course, to isolate correlative receptors or ligands. Where the protein binds or potentially binds to another protein (such as, for example, in a receptor-ligand interaction), the protein can be used to identify the other protein with which binding occurs or to identify inhibitors of the binding interaction. Proteins involved in these binding interactions can also be used to screen for peptide or small molecule inhibitors or agonists of the binding interaction.

Any or all of these research utilities are capable of being developed into reagent grade or kit format for commercialization as research products.

Methods for performing the uses listed above are well known to those skilled in the art. References disclosing such methods include without limitation "Molecular Cloning: A Laboratory Manual", 2d ed., Cold Spring Harbor Laboratory Press, Sambrook, J., E.F. Fritsch and T. Maniatis eds., 1989, and "Methods in Enzymology: Guide to Molecular Cloning Techniques", Academic Press, Berger, S.L. and A.R. Kimmel eds., 1987.

### Nutritional Uses

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Polynucleotides and proteins of the present invention can also be used as nutritional sources or supplements. Such uses include without limitation use as a protein or amino acid supplement, use as a carbon source, use as a nitrogen source and use as a source of carbohydrate. In such cases the protein or polynucleotide of the invention can be added to the feed of a particular organism or can be

administered as a separate solid or liquid preparation, such as in the form of powder, pills, solutions, suspensions or capsules. In the case of microorganisms, the protein or polynucleotide of the invention can be added to the medium in or on which the microorganism is cultured.

# Cytokine and Cell Proliferation/Differentiation Activity

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A protein of the present invention may exhibit cytokine, cell proliferation (either inducing or inhibiting) or cell differentiation (either inducing or inhibiting) activity or may induce production of other cytokines in certain cell populations. Many protein factors discovered to date, including all known cytokines, have exhibited activity in one or more factor dependent cell proliferation assays, and hence the assays serve as a convenient confirmation of cytokine activity. The activity of a protein of the present invention is evidenced by any one of a number of routine factor dependent cell proliferation assays for cell lines including, without limitation, 32D, DA2, DA1G, T10, B9, B9/11, BaF3, MC9/G, M+ (preB M+), 2E8, RB5, DA1, 123, T1165, HT2, CTLL2, TF-1, Mo7e and CMK.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for T-cell or thymocyte proliferation include without limitation those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. J. 137:3494-3500, 1986; Bertagnolli et al., Immunol. Bertagnolli et al., 145:1706-1712, 1990; Cellular

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Immunology 133:327-341, 1991; Bertagnolli, et al., J.
Immunol. 149:3778-3783, 1992; Bowman et al., J. Immunol.
152: 1756-1761, 1994.

Assays for cytokine production and/or proliferation of spleen cells, lymph node cells or thymocytes include, without limitation, those described in: Polyclonal T cell stimulation, Kruisbeek, A.M. and Shevach, E.M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.12.1-3.12.14, John Wiley and Sons, Toronto. 1994; and Measurement of mouse and human Interferon  $\gamma$ , Schreiber, R.D. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.8.1-6.8.8, John Wiley and Sons, Toronto. 1994.

Assays for proliferation and differentiation of include, without hematopoietic and lymphopoietic cells limitation, those described in: Measurement of Human and Murine Interleukin 2 and Interleukin 4, Bottomly, K., Davis, L.S. and Lipsky, P.E. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.3.1-6.3.12, John Wiley and Sons, Toronto. 1991; deVries et al., J. Exp. Med. 173:1205-1211, 1991: Moreau et al., Nature 336:690-692, Greenberger et al., Proc. Natl. Acad. Sci. U.S.A. 80:2931-2938, 1983; Measurement of mouse and human interleukin 6-Nordan, R. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 6.6.1-6.6.5, John Wiley and Sons, Toronto. 1991; Smith et al., Proc. Natl. Acad. Sci. U.S.A. 83:1857-1861, 1986; Measurement of human Interleukin 11 -Bennett, F., Giannotti, J., Clark, S.C. and Turner, K. J. In Current Protocols in Immunology. J.E.e.a. Coligan eds. pp. 6.15.1 John Wiley and Sons, Toronto. 1991; Measurement of mouse and human Interleukin 9 - Ciarletta, A., Giannotti, J., Clark, S.C. and Turner, K.J. Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp.

6.13.1, John Wiley and Sons, Toronto. 1991.

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Assays for T-cell clone responses to antigens (which will identify, among others, proteins that affect APC-T cell interactions as well as direct T-cell effects by measuring proliferation and cytokine production) include, without those described in: Current Protocols limitation, Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function; Chapter 6, Cytokines and their cellular receptors; Chapter 7, Immunologic studies in Humans); Weinberger et al., Proc. Natl. Acad. Sci. 77:6091-6095, 1980; Weinberger et al., Eur. J. 11:405-411, 1981; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988.

### Immune Stimulating or Suppressing Activity

A protein of the present invention may also exhibit immune stimulating or immune suppressing activity, including without limitation the activities for which assays are described herein. A protein may be useful in the treatment of various immune deficiencies and disorders (including immunodeficiency severe combined (SCID)), e.q., regulating (up or down) growth and proliferation of T and/or B lymphocytes, as well as effecting the cytolytic activity of NK cells and other cell populations. These deficiencies may be genetic or be caused by viral (e.g., HIV) as well as bacterial orfungal infections, or may result from autoimmune disorders. More specifically, infectious diseases causes by viral, bacterial, fungal or other infection may be treatable using a protein of the present invention, including infections by HIV, hepatitis viruses, herpesviruses, mycobacteria, Leishmania spp., malaria spp.

and various fungal infections such as candidiasis. Of course, in this regard, a protein of the present invention may also be useful where a boost to the immune system generally may be desirable, i.e., in the treatment of cancer.

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Autoimmune disorders which may be treated using a protein of the present invention include, for example, connective tissue disease, multiple sclerosis, systemic rheumatoid arthritis, autoimmune erythematosus, pulmonary inflammation, Guillain-Barre syndrome, autoimmune thyroiditis, insulin dependent diabetes mellitis, myasthenia graft-versus-host disease and autoimmune gravis, Such a protein of the present inflammatory eye disease. invention may also to be useful in the treatment of allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems. conditions, in which immune suppression desired (including, for example, organ transplantation), may also be treatable using a protein of the present invention.

Using the proteins of the invention it may also be possible to immune responses, in a number of ways. regulation may be in the form of inhibiting or blocking an already in progress or response may preventing the induction of an immune response. activated T cells may be inhibited functions of cell responses or by inducing specific suppressing T Immunosuppression of T cell tolerance in T cells, or both. generally an active, non-antigen-specific, responses is process which requires continuous exposure of the T cells to Tolerance, which involves inducing the suppressive agent. non-responsiveness or anergy in T cells, is distinguishable from immunosuppression in that it is generally antigenspecific and persists after exposure to the tolerizing agent

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WO 00/05367 PCT/JP99/03929

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has ceased. Operationally, tolerance can be demonstrated by the lack of a T cell response upon reexposure to specific antigen in the absence of the tolerizing agent.

Down regulating or preventing one or more antigen functions (including without limitation B lymphocyte antigen functions (such as , for example, B7)), e.g., preventing high level lymphokine synthesis by activated T cells, will useful in situations of tissue, skin transplantation and in graft-versus-host disease (GVHD). For example, blockage of T cell function should result in in tissue reduced tissue destruction transplantation. tissue transplants, rejection Typically, in transplant is initiated through its recognition as foreign by T cells, followed by an immune reaction that destroys the transplant. The administration of a molecule which inhibits or blocks interaction of a B7 lymphocyte antigen with its natural ligand(s) on immune cells (such as a soluble, monomeric form of a peptide having B7-2 activity alone or in conjunction with a monomeric form of a peptide having an activity of another B lymphocyte antigen (e.g., B7-1, B7-3) or blocking antibody), prior to transplantation can lead to the binding of the molecule to the natural ligand(s) on the without transmitting the corresponding cells immune Blocking B lymphocyte costimulatory signal. function in this matter prevents cytokine synthesis immune cells, such as T cells, and thus acts as immunosuppressant. Moreover, the lack of costimulation may also be sufficient to anergize the T cells, thereby inducing tolerance in a subject. Induction of long-term tolerance by lymphocyte antigen-blocking reagents may avoid necessity of repeated administration of these blocking To achieve sufficient immunosuppression reagents.

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tolerance in a subject, it may also be necessary to block the function of a combination of B lymphocyte antigens.

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efficacy of particular blocking reagents preventing organ transplant rejection or GVHD can assessed using animal models that are predictive of efficacy Examples of appropriate systems which can be in humans. allogeneic cardiac grafts in include xenogeneic pancreatic islet cell grafts in mice, both of which have been used to examine the immunosuppressive effects of CTLA4Iq fusion proteins in vivo as described in Lenschow et al., Science 257:789-792 (1992) and Turka et al., Proc. Natl. Acad. Sci USA, 89:11102-11105 (1992). addition, murine models of GVHD (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 846-847) can be used to determine the effect of blocking B lymphocyte antigen function in vivo on the development of that disease.

Blocking antigen function may also be therapeutically useful for treating autoimmune diseases. Many autoimmune disorders are the result of inappropriate activation of T cells that are reactive against self tissue and which promote the production of cytokines and autoantibodies involved in the pathology of the diseases. Preventing the activation of autoreactive T cells may reduce or eliminate Administration of reagents which block disease symptoms. costimulation of T cells by disrupting receptor:ligand interactions of B lymphocyte antigens can be used to inhibit T cell activation and prevent production of autoantibodies or T cell-derived cytokines which may be involved in the disease process. Additionally, blocking reagents may induce antigen-specific tolerance of autoreactive T cells which could lead to long-term relief from the disease. efficacy of blocking reagents in preventing or alleviating

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autoimmune disorders can be determined using a number of of well-characterized animal models human autoimmune Examples include murine experimental autoimmune diseases. encephalitis, systemic lupus erythmatosis in MRL/lpr/lpr hybrid mice, murine autoimmune collagen mice or NZB arthritis, diabetes mellitus in NOD mice and BB rats, and murine experimental myasthenia gravis (see Paul ed., Fundamental Immunology, Raven Press, New York, 1989, pp. 840-856).

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Upregulation of an antigen function (preferably a B lymphocyte antigen function), as a means of up regulating also be useful responses, may in therapy. immune Upregulation of immune responses may be in the form of enhancing an existing immune response or eliciting initial immune response. For example, enhancing an immune response through stimulating B lymphocyte antigen function may be useful in cases of viral infection. In addition, systemic viral diseases such as influenza, the commoncold, and encephalitis might be alleviated by the administration of stimulatory forms of B lymphocyte antigens systemically.

Alternatively, anti-viral immune responses may be enhanced in an infected patient by removing T cells from the patient, costimulating the T cells in vitro with viral antigen-pulsed APCs either expressing a peptide of the present invention or together with a stimulatory form of a soluble peptide of the present invention and reintroducing the in vitro activated T cells into the patient. Another method of enhancing anti-viral immune responses would be to isolate infected cells from a patient, transfect them with a nucleic acid encoding a protein of the present invention as described herein such that the cells express all or a portion of the protein on their surface, and reintroduce the

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transfected cells into the patient. The infected cells would now be capable of delivering a costimulatory signal to, and thereby activate, T cells in vivo.

In another application, up regulation or enhancement of antiquen function (preferably B lymphocyte antiquen function) may be useful in the induction of tumor immunity. sarcoma, melanoma, lymphoma, (e.g., leukemia. neuroblastoma, carcinoma) transfected with a nucleic acid encoding at least one peptide of the present invention can be administered to a subject to overcome tumor-specific tolerance in the subject. If desired, the tumor cell can be transfected to express a combination of peptides. tumor cells obtained from a patient can be example, transfected ex vivo with an expression vector directing the expression of a peptide having B7-2-like activity alone, or in conjunction with a peptide having B7-1-like activity and/or B7-3-like activity. The transfected tumor cells are returned to the patient to result in expression of the peptides on the surface of the transfected Alternatively, gene therapy techniques can be used to target a tumor cell for transfection in vivo.

The presence of the peptide of the present invention having the activity of a B lymphocyte antigen(s) on the surface of the tumor cell provides the necessarv costimulation signal to T cells to induce a T cell mediated immune response against the transfected tumor cells. addition, tumor cells which lack MHC class I or MHC class II molecules, or which fail to reexpress sufficient amounts of MHC class I or MHC class II molecules, can be transfected with nucleic acid encoding all or a portion of (e.g., a cytoplasmic-domain truncated portion) of an MHC class I  $\alpha$ chain protein and , microglobulin protein or an MHC class

chain protein and an MHC class II chain protein to II thereby express MHC class I or MHC class II proteins on the cell surface. Expression of the appropriate class I or class II MHC in conjunction with a peptide having the activity of a B lymphocyte antigen (e.g., B7-1, B7-2, B7-3) induces a T cell mediated immune response against the transfected tumor cell. Optionally, a gene encoding an antisense construct which blocks expression of an MHC class II associated protein, such as the invariant chain, can also be cotransfected with a DNA encoding a peptide having the activity of a B lymphocyte antigen to promote presentation of tumor associated antigens and induce tumor specific Thus, the induction of a T cell mediated immune immunity. response in a human subject may be sufficient to overcome tumor-specific tolerance in the subject.

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The activity of a protein of the invention may, among other means, be measured by the following methods:

for thymocyte Suitable assays orsplenocyte cytotoxicity include, without limitation, those described Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Herrmann et al., Proc. Natl. Acad. Sci. USA 78:2488-2492, 1981; Herrmann et al., J. Immunol. 128:1968-1974, 1982; Handa et al., J. Immunol. 135:1564-1572, 1985; Takai et al., J. Immunol. 137:3494-3500, 1986; Bowmanet al., J.

Virology 61:1992-1998; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., Cellular Immunology 133:327-341, 1991; Brown et al., J. Immunol. 153:3079-3092, 1994.

Assays for T-cell-dependent immunoglobulin responses and isotype switching (which will identify, among others, proteins that modulate T-cell dependent antibody responses and that affect Th1/Th2 profiles) include, without limitation, those described in: Maliszewski, J. Immunol. 144:3028-3033, 1990; and Assays for B cell function: In vitro antibody production, Mond, J.J. and Brunswick, M. In Current Protocols in Immunology. J.E.e.a. Coligan eds. Vol 1 pp. 3.8.1-3.8.16, John Wiley and Sons, Toronto. 1994.

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Mixed lymphocyte reaction (MLR) assays (which will identify, among others, proteins that generate predominantly Th1 and CTL responses) include, without limitation, those described in: Current Protocols in Immunology, Ed by J. E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 3, In Vitro assays for Mouse Lymphocyte Function 3.1-3.19; Chapter 7, Immunologic studies in Humans); Takai et al., J. Immunol. 137:3494-3500, 1986; Takai et al., J. Immunol. 140:508-512, 1988; Bertagnolli et al., J. Immunol. 149:3778-3783, 1992.

Dendritic cell-dependent assays (which will identify, among others, proteins expressed by dendritic cells that activate naive T-cells) include, without limitation, those described in: Guery et al., J. Immunol. 134:536-544, 1995; Inaba et al., Journal of Experimental Medicine 173:549-559, 1991; Macatonia et al., Journal of Immunology 154:5071-5079, 1995; Porgador et al., Journal of Experimental Medicine 182:255-260, 1995; Nair et al., Journal of Virology 67:4062-4069, 1993; Huang et al., Science 264:961-965,

1994; Macatonia et al., Journal of Experimental Medicine 169:1255-1264, 1989; Bhardwaj et al., Journal of Clinical Investigation 94:797-807, 1994; and Inaba et al., Journal of Experimental Medicine 172:631-640, 1990.

Assays for lymphocyte survival/apoptosis (which will identify, among others, proteins that prevent apoptosis after superantigen induction and proteins that regulate lymphocyte homeostasis) include, without limitation, those described in: Darzynkiewicz et al., Cytometry 13:795-808, 1992; Gorczyca et al., Leukemia 7:659-670, 1993; Gorczyca et al., Cancer Research 53:1945-1951, 1993; Itoh et al., Cell Journal 66:233-243, 1991; Zacharchuk, of Immunology 145:4037-4045, 1990; Zamai et al., Cytometry 14:891-897, 1993; Gorczyca et al., International Journal of Oncology 1:639-648, 1992.

Assays for proteins that influence early steps of T-cell commitment and development include, without limitation, those described in: Antica et al., Blood 84:111-117, 1994; Fine et al., Cellular Immunology 155:111-122, 1994; Galy et al., Blood 85:2770-2778, 1995; Toki et al., Proc. Nat. Acad Sci. USA 88:7548-7551, 1991.

### Hematopoiesis Regulating Activity

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A protein of the present invention may be useful in regulation of hematopoiesis and, consequently, treatment of myeloid or lymphoid cell deficiencies. marginal biological activity in support of colony forming factor-dependent cell lines indicates cells or of involvement in regulating hematopoiesis, e.g. in supporting the growth and proliferation of erythroid progenitor cells alone or in combination with other cytokines, thereby indicating utility, for example, in treating various anemias or for use in conjunction with irradiation/chemotherapy to

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stimulate the production of erythroid precursors and/or erythroid cells; in supporting the growth and proliferation granulocytes as of mveloid cells such and (i.e., traditional monocytes/macrophages CSF activity) useful, for example, in conjunction with chemotherapy to prevent or treat consequent myelo-suppression; in supporting growth and proliferation of megakaryocytes consequently of platelets thereby allowing prevention or treatment of various platelet disorders such thrombocytopenia, and generally for use in place of or complimentary to platelet transfusions; and/or in supporting the growth and proliferation of hematopoietic stem cells which are capable of maturing to any and all of the abovementioned hematopoietic cells and therefore find therapeutic utility in various stem cell disorders (such as those usually treated with transplantation, including, without aplastic limitation, anemia and paroxysmal nocturnal hemoglobinuria), as well as in repopulating the stem cell compartment post irradiation/chemotherapy, either in-vivo or ex-vivo in conjunction with bone (i.e., marrow transplantation or with peripheral progenitor cell transplantation (homologous or heterologous)) as normal cells or genetically manipulated for gene therapy.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for proliferation and differentiation of various hematopoietic lines are cited above.

Assays for embryonic stem cell differentiation (which will identify, among others, proteins that influence embryonic differentiation hematopoiesis) include, without limitation, those described in: Johansson et al. Cellular Biology 15:141-151, 1995; Keller et al., Molecular and

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Cellular Biology 13:473-486, 1993; McClanahan et al., Blood 81:2903-2915, 1993.

Assays for stem cell survival and differentiation (which will identify, among others, proteins that regulate lympho-hematopoiesis) include, without limitation, those described in: Methylcellulose colony forming Freshney, M.G. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 265-268, Wiley-Liss, Inc., New York, NY. 1994; Hirayama et al., Proc. Natl. Acad. Sci. 1992; Primitive hematopoietic colony USA 89:5907-5911, forming cells with high proliferative potential, McNiece, I.K. and Briddell, R.A. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 23-39, Wiley-Liss, Inc., New York, NY. 1994; Neben et al., Experimental Hematology 22:353-359, 1994; Cobblestone area forming cell assay, Ploemacher, R.E. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 1-21, Wiley-Liss, Inc., New York, NY. 1994; Long term bone marrow cultures in the presence of stromal cells, Spooncer, E., Dexter, M. and Allen, T. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 163-179, Wiley-Liss, Inc., New York, NY. 1994; Long term culture initiating cell assay, Sutherland, H.J. In Culture of Hematopoietic Cells. R.I. Freshney, et al. eds. Vol pp. 139-162, Wiley-Liss, Inc., New York, NY. 1994.

#### Tissue Growth Activity

A protein of the present invention also may have utility in compositions used for bone, cartilage, tendon, ligament and/or nerve tissue growth or regeneration, as well as for wound healing and tissue repair and replacement, and in the treatment of burns, incisions and ulcers.

A protein of the present invention, which induces cartilage and/or bone growth in circumstances where bone is

31

not normally formed, has application in the healing of bone fractures and cartilage damage or defects in humans and other animals. Such a preparation employing a protein of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery.

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A protein of this invention may also be used in the treatment of periodontal disease, and in other tooth repair Such agents may provide an environment to processes. attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of boneforming cells. A protein of the invention may also be useful in the treatment of osteoporosis or osteoarthritis, such as through stimulation of bone and/or cartilage repair blocking inflammation or processes of by destruction (collagenase activity, osteoclast activity, etc.) mediated by inflammatory processes.

Another category of tissue regeneration activity that may be attributable to the protein of the present invention is tendon/ligament formation. A protein of the present invention, which induces tendon/ligament-like tissue or other tissue formation in circumstances where such tissue is not normally formed, has application in the healing of tendon or ligament tears, deformities and other tendon or ligament defects in humans and other animals. Such a preparation employing a tendon/ligament-like tissue inducing protein may have prophylactic use in preventing damage to tendon or ligament tissue, as well as use in the improved fixation of tendon or ligament to bone or other tissues, and

32

in repairing defects to tendon or ligament tissue. De novo tendon/ligament-like tissue formation induced by composition of the present invention contributes to the repair of congenital, trauma induced, or other tendon or ligament defects of other origin, and is also useful in cosmetic plastic surgery for attachment or repair of tendons or ligaments. The compositions of the present invention may provide an environment to attract tendon or ligament-forming cells, stimulate growth of tendon- or ligament-forming cells, induce differentiation of progenitors of tendonligament-forming cells, or induce growth of tendon/ligament cells or progenitors ex vivo for return in vivo to effect tissue repair. The compositions of the invention may also be useful in the treatment of tendinitis, carpal tunnel syndrome and other tendon or ligament defects. compositions may also include an appropriate matrix and/or sequestering agent as a carrier as is well known in the art.

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The protein of the present invention may also be useful for proliferation of neural cells and for regeneration of nerve and brain tissue, i.e. for the treatment of central and peripheral nervous system diseases and neuropathies, as well as mechanical and traumatic disorders, which involve degeneration, death or trauma to neural cells or nerve More specifically, a protein may be used in the treatment of diseases of the peripheral nervous system, such as peripheral nerve injuries, peripheral neuropathy and localized neuropathies, and central nervous system diseases, such as Alzheimer's, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome. Further conditions which may be treated in accordance with the present invention include mechanical and traumatic disorders, such as spinal cord disorders, head

PCT/JP99/03929

trauma and cerebrovascular diseases such as stroke. Peripheral neuropathies resulting from chemotherapy or other medical therapies may also be treatable using a protein of the invention.

Proteins of the invention may also be useful to promote better or faster closure of non-healing wounds, including without limitation pressure ulcers, ulcers associated with vascular insufficiency, surgical and traumatic wounds, and the like.

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It is expected that a protein of the present invention may also exhibit activity for generation or regeneration of other tissues, such as organs (including, for example, pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac) and vascular (including vascular endothelium) tissue, or for promoting the growth of cells comprising such tissues. Part of the desired effects may be by inhibition or modulation of fibrotic scarring to allow normal tissue to regenerate. A protein of the invention may also exhibit angiogenic activity.

A protein of the present invention may also be useful for gut protection or regeneration and treatment of lung or liver fibrosis, reperfusion injury in various tissues, and conditions resulting from systemic cytokine damage.

A protein of the present invention may also be useful for promoting or inhibiting differentiation of tissues described above from precursor tissues or cells; or for inhibiting the growth of tissues described above.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assays for tissue generation activity include, without limitation, those described in: International Patent Publication No. W095/16035 (bone, cartilage, tendon);

International Patent Publication No. W095/05846 neuronal); International Patent Publication No. W091/07491 (skin, endothelium).

Assays for wound healing activity include, without limitation, those described in: Winter, Epidermal Wound Healing, pps. 71-112 (Maibach, HI and Rovee, DT, eds.), Year Book Medical Publishers, Inc., Chicago, as modified by Eaglstein and Mertz, J. Invest. Dermatol 71:382-84 (1978).

#### Activin/Inhibin Activity

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A protein of the present invention may also exhibit inhibin-related activities. Inhibins activinor characterized by their ability to inhibit the release of follicle stimulating hormone (FSH), while activins and are characterized by their ability to stimulate the release of follicle stimulating hormone (FSH). Thus, a protein of the present invention, alone or in heterodimers with a member of family, may be useful as a contraceptive based the inhibin on the ability of inhibins to decrease fertility in female and decrease spermatogenesis in male mammals. mammals Administration of sufficient amounts of other inhibins can induce infertility in these mammals. Alternatively, the protein of the invention, as a homodimer or as a heterodimer with other protein subunits of the inhibin- group, may be useful as a fertility inducing therapeutic, based upon the ability of activin molecules in stimulating FSH release from cells of the anterior pituitary. See, for example, United States Patent 4,798,885. A protein of the invention may also be useful for advancement of the onset of fertility in sexually immature mammals, so as to increase the lifetime reproductive performance of domestic animals such as cows, sheep and pigs.

The activity of a protein of the invention may, among

other means, be measured by the following methods:

Assays for activin/inhibin activity include, without limitation, those described in: Vale et al., Endocrinology 91:562-572, 1972; Ling et al., Nature 321:779-782, 1986; Vale et al., Nature 321:776-779, 1986; Mason et al., Nature 318:659-663, 1985; Forage et al., Proc. Natl. Acad. Sci. USA 83:3091-3095, 1986.

# Chemotactic/Chemokinetic Activity

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A protein of the present invention may have chemotactic or chemokinetic activity (e.g., act as a chemokine) for including, for mammalian cells, example, monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells. Chemotactic and chemokinetic proteins can be used to mobilize or attract a desired cell population to a desired site of action. Chemotactic or chemokinetic proteins provide particular advantages in treatment of wounds and other trauma to tissues, as well as in treatment of localized infections. attraction of lymphocytes, monocytes example, neutrophils to tumors or sites of infection may result in improved immune responses against the tumor or infecting agent.

A protein or peptide has chemotactic activity for a particular cell population if it can stimulate, directly or indirectly, the directed orientation or movement of such cell population. Preferably, the protein or peptide has the ability to directly stimulate directed movement of cells. Whether a particular protein has chemotactic activity for a population of cells can be readily determined by employing such protein or peptide in any known assay for cell chemotaxis.

The activity of a protein of the invention may, among

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other means, be measured by the following methods:

Assays for chemotactic activity (which will identify proteins that induce or prevent chemotaxis) consist of assays that measure the ability of a protein to induce the migration of cells across a membrane as well as the ability of a protein to induce the adhesion of one cell population to another cell population. Suitable assays for movement and adhesion include, without limitation, those described in: Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 6.12, Measurement of alpha and beta Chemokines 6.12.1-6.12.28; Taub et al. J. Clin. Invest. 95:1370-1376, 1995; Lind et al. APMIS 103:140-146, 1995; Muller et al Eur. J. Immunol. 25: 1744-1748; Gruber et al. J. of Immunol. 152:5860-5867, 1994; Johnston et al. J. of Immunol. 153: 1762-1768, 1994.

# Hemostatic and Thrombolytic Activity

A protein of the invention may also exhibit hemostatic or thrombolytic activity. As a result, such a protein is expected to be useful in treatment of various coagulation disorders (includinghereditary disorders, such as hemophilias) or to enhance coagulation and other hemostatic events in treating wounds resulting from trauma, surgery or other causes. A protein of the invention may also be useful for dissolving or inhibiting formation of thromboses and for treatment and prevention of conditions resulting therefrom (such as, for example, infarction of cardiac and central nervous system vessels (e.g., stroke).

The activity of a protein of the invention may, among other means, be measured by the following methods:

Assay for hemostatic and thrombolytic activity include,

without limitation, those described in: Linet et al., J. 1986; Clin. Pharmacol. 26:131-140, Burdick et al.. 1987; Thrombosis Res. 45:413-419, Humphrey al., Fibrinolysis 5:71-79 (1991); Schaub, Prostaglandins 35:467-474. 1988.

# Receptor/Ligand Activity

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A protein of the present invention may also demonstrate activity as receptors, receptor ligands or inhibitors or agonists of receptor/ligand interactions. Examples of such receptors and ligands include, without limitation, cytokine receptors and their ligands, receptor kinases and their ligands, receptor phosphatases and their ligands, receptors involved in cell-cell interactions and their (including without limitation, cellular adhesion molecules selectins, integrins and their ligands) receptor/ligand pairs involved in antigen presentation, antigen recognition and development of cellular and humoral Receptors and ligands are also useful immune responses). for screening of potential peptide or small molecule inhibitors of the relevant receptor/ligand interaction. the present invention protein of (including, without limitation, fragments of receptors and ligands) receptor/ligand themselves be useful as inhibitors of interactions.

The activity of a protein of the invention may, among other means, be measured by the following methods:

Suitable assays for receptor-ligand activity include without limitation those described in:Current Protocols in Immunology, Ed by J.E. Coligan, A.M. Kruisbeek, D.H. Margulies, E.M. Shevach, W.Strober, Pub. Greene Publishing Associates and Wiley-Interscience (Chapter 7.28, Measurement of Cellular Adhesion under static conditions 7.28.1-7.28.22),

Takai et al., Proc. Natl. Acad. Sci. USA 84:6864-6868, 1987; Bierer et al., J. Exp. Med. 168:1145-1156, 1988; Rosenstein et al., J. Exp. Med. 169:149-160 1989; Stoltenborg et al., J. Immunol. Methods 175:59-68, 1994; Stitt et al., Cell 80:661-670, 1995.

# Anti-Inflammatory Activity

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Proteins of the present invention may also exhibit anti-inflammatory activity. The anti-inflammatory activity may be achieved by providing a stimulus to cells involved in the inflammatory response, by inhibiting or promoting cellcell interactions (such as, for example, cell adhesion), by inhibiting or promoting chemotaxis of cells involved in the inflammatory process, inhibiting or promoting extravasation, or by stimulating or suppressing production of other factors which more directly inhibit or promote an inflammatory response. Proteins exhibiting such activities can be used to treat inflammatory conditions including chronic or acute conditions), including without limitation inflammation associated with infection (such as septic shock, sepsis or systemic inflammatory response syndrome (SIRS)), ischemia-reperfusion injury, endotoxin lethality, arthritis, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine-induced lung injury, inflammatory bowel disease, Crohn's disease or resulting from over production of ytokines such as TNF or IL-1. Proteins of the invention may also be useful to treat anaphylaxis and hypersensitivity to an antigenic substance or material.

# Tumor Inhibition Activity

In addition to the activities described above for immunological treatment or prevention of tumors, a protein of the invention may exhibit other anti-tumor activities. A

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protein may inhibit tumor growth directly or indirectly (such as, for example, via ADCC). A protein may exhibit its tumor inhibitory activity by acting on tumor tissue or tumor precursor tissue, by inhibiting formation of tissues necessary to support tumor growth (such as, for example, by inhibiting angiogenesis), by causing production of other factors, agents or cell types which inhibit tumor growth, or by suppressing, eliminating or inhibiting factors, agents or cell types which promote tumor growth

# 10 Other Activities

A protein of the invention may also exhibit one or more additional activities of following or inhibiting the growth, infection or function of, or killing, infectious agents, including, without limitation, bacteria, viruses, fungi and other parasites; effecting (suppressing or enhancing) bodily characteristics, including, without limitation, height, weight, hair color, eye color, skin, fat to lean ratio or other tissue pigmentation, or organ or body as, shape (such for example, breast size or augmentation or diminution, change in bone form or shape); effecting biorhythms or caricadic cycles or rhythms; effecting the fertility of male or female subjects: effecting the metabolism, catabolism, anabolism, processing, utilization, storage or elimination of dietary fat, lipid, protein, carbohydrate, vitamins, minerals, cofactors or component(s); other nutritional factors or effecting behavioral characteristics, including, without limitation, appetite, libido, stress, cognition (including cognitive disorders), depression (including depressive disorders) and violent behaviors; providing analgesic effects or other pain reducing effects; promoting differentiation and growth of

embryonic stem cells in lineages other than hematopoietic lineages; hormonal or endocrine activity; in the case of enzymes, correcting deficiencies of the enzyme and treating deficiency-related diseases; treatment of hyperproliferative disorders (such as, for example, psoriasis); immunoglobulin-like activity (such as, for example, the ability to bind antigens or complement); and the ability to act as an antigen in a vaccine composition to raise an immune response against such protein or another material or entity which is cross-reactive with such protein.

# Examples

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The present invention is specifically illustrated in more detail by the following Examples, but Examples are not intended to restrict the present invention. The basic operations with regard to the recombinant DNA and the enzymatic reactions were carried out according to the literature ["Molecular Cloning. A Laboratory Manual", Cold Spring Harbor Laboratory, 1989]. Unless otherwise stated, restrictive enzymes and a variety of modification enzymes to be used were those available from Takara Shuzo. The buffer compositions and the reaction conditions for each of the enzyme reactions were as described in the manufacturer's instructions. The cDNA synthesis was carried out according to the literature [Kato, S. et al., Gene 150: 243-250 (1994)].

(1) Selection of cDNAs Encoding Proteins Having Hydrophobic Domains

The cDNA library of fibrosarcoma cell line HT-1080 (WO98/11217), the cDNA library of osteosarcoma cell line Saos-2 (WO97/33993), the cDNA library of osteosarcoma cell line U-2 OS (WO98/21328), the cDNA library of epidermoid

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carcinoma cell line KB (WO98/11217), the cDNA library of tissues of stomach cancer delivered by the operation (WO98/21328), the cDNA library of liver tissue delivered by the operation (WO98/21328), and were used for the cDNA libraries. Full-length cDNA clones were selected respective libraries and the whole base sequences thereof were determined to construct a homo-protein cDNA consisting of the full-length CDNA clones. The hydrophobicity/hydrophilicity profiles were determined for proteins encoded by the full-length cDNA clones registered in the homo-protein cDNA bank by the Kyte-Doolittle method [Kyte, J. & Doolittle, R. F., J. Mol. Biol. 157: 105-132 (1982)] to examine the presence or absence of a hydrophobic region. Any clone that has a hydrophobic region being putative as a secretory signal or a transmembrane domain in the amino acid sequence of the encoded protein was

#### (2) Protein Synthesis by In Vitro Translation

selected as a clone candidate.

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The plasmid vector bearing the cDNA of the present invention was used for in vitro transcription/translation with a  $T_NT$  rabbit reticulocyte lysate kit (Promega). In this case, [ $^{15}S$ ]methionine was added to label the expression product with a radioisotope. Each of the reactions was carried out according to the protocols attached to the kit. Two micrograms of the plasmid was subjected to the reaction at 30°C for 90 minutes in the reaction solution of a total volume of 25  $\mu$ l containing 12.5  $\mu$ l  $\mu$  of  $T_NT$  rabbit reticulocyte lysate, 0.5  $\mu$ l of a buffer solution (attached to the kit), 2  $\mu$ l of an amino acid mixture (without methionine), 2  $\mu$ l of [ $^{35}S$ ]methionine (Amersham) (0.37 MBq/ $\mu$ l), 0.5  $\mu$ l of T7 RNA polymerase, and 20 U of RNasin. Also, an experiment in the presence of a membrane system was carried

out by adding to this reaction system 2.5  $\mu$ l of a canine pancreas microsome fraction (Promega). To 3  $\mu$ l of the resulting reaction solution was added 2  $\mu$ l of the SDS sampling buffer (125 mM Tris-hydrochloric acid buffer, pH 6.8, 120 mM 2-mercaptoethanol, 2% SDS solution, 0.025% bromophenol blue, and 20% glycerol) and the resulting mixture was heated at 95°C for 3 minutes and then subjected to SDS-polyacrylamide gel electrophoresis. The molecular weight of the translation product was determined by carrying out the autoradiography.

# (3) Expression by COS7

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Escherichia coli cells bearing the expression vector for the protein of the present invention was incubated at  $37^{\circ}\text{C}$  for 2 hours in 2 ml of the 2xYT culture medium containing  $100~\mu\text{g/ml}$  of ampicillin, the helper phage M13K07 ( $50~\mu$ 1) was added, and the incubation was continued at  $37^{\circ}\text{C}$  overnight. A supernatant separated by centrifugation underwent precipitation with polyethylene glycol to obtain single-stranded phage particles. These particles were suspended in  $100~\mu\text{l}$  of 1 mM Tris-0.1 mM EDTA, pH 8 (TE).

The cultured cells derived from simian kidney, COS7, were incubated at 37°C in the presence of 5% CO<sub>2</sub> in the Dulbecco's modified Eagle's culture medium (DMEM) containing 10% fetal calf serum. Into a 6-well plate (Nunc, well diameter: 3 cm) were inoculated with 1 x  $10^5$  COS7 cells and incubation was carried out at 37°C for 22 hours in the presence of 5% CO<sub>2</sub>. After the culture medium was removed, the cell surface was washed with a phosphate buffer solution and then washed again with DMEM containing 50 mM Trishydrochloric acid (pH 7.5) (TDMEM). To the resulting cells was added a suspension of 1  $\mu$ l of the single-stranded phage suspension, 0.6 ml of the DMEM culture medium, and 3  $\mu$ l of

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TRANSFECTAMTM (IBF) and the resulting mixture was incubated at 37°C for 3 hours in the presence of 5% CO2. After the sample solution was removed, the cell surface was washed with TDMEM, 2 ml per well of DMEM containing 10% fetal calf serum was added, and the incubation was carried out at 37°C for 2 days in the presence of 5% CO2. After the culture replaced by a culture medium containing [35S]cystine or [35S]methionine, the incubation was carried out for one hour. After the culture medium and the cells were separated by centrifugation, proteins in the culture the cell-membrane fraction medium fraction and subjected to SDS-PAGE.

# (4) Clone Examples <HP01550> (SEQ ID Nos. 1, 11, and 21)

Determination of the whole base sequence of the cDNA insert of clone HP01550 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 65-bp 5'-untranslated region, a 378-bp ORF, and a 67-bp 3'untranslated region. The ORF codes for a protein consisting of 125 amino acid residues and there existed one putative domain. Figure 1 depicts the transmembrane hydrophobicity/hydrophilicity profile, obtained by the Kytethe present protein. Doolittle method, of translation resulted in formation of a translation product of 15 kDa that was almost identical with the molecular weight of 13,825 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Caenorhabditis elegans hypothetical protein F45G2.c (GenBank Accession No. Z93382). Table 2 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C.

elegans hypothetical protein F45G2.c (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 44.5% in the entire region.

#### Table 2

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA338859) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02593> (SEQ ID Nos. 2, 12, and 22)

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Determination of the whole base sequence of the cDNA insert of clone HP02593 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 103-bp 5'-untranslated region, a 396-bp ORF,

and a 198-bp 3'-untranslated region. The ORF codes for a protein consisting of 131 amino acid residues and there existed four putative transmembrane domains at the C-terminus. Figure 2 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of a high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to a human OB-R gene-related protein (EMBL Accession No. Y12670). Table 3 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human OB-R gene-related protein (OB). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 67.9% in the entire region.

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#### Table 3

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA306490) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP10195> (SEQ ID Nos. 3, 13, and 23)

Determination of the whole base sequence of the cDNA insert of clone HP10195 obtained from cDNA library of human fibrosarcoma HT-1080 revealed the structure consisting of a 286-bp 5'-untranslated region, a 729-bp ORF, and a 604-bp The ORF codes for a 3'-untranslated region. consisting of 242 amino acid residues and there existed one putative transmembrane domain at the C-terminus. Figure 3 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 32 kDa that was somewhat larger than the molecular weight of 27,300 predicted from the ORF. When expressed in COS7 cells, an expression product of about 21 kDa was observed in the supernatant fraction and the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has revealed the registration of sequences that were similar to the Aplysia VAP-33 (SWISS-PROT Accession No. P53173). Table 4 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the Aplysia VAP-33 (AP). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the

present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 46.5% in the entire region.

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#### Table 4

HP MAKHEQILVLDPPTDLKFKGPFTDVVTTNLKLRNPSDRKVCFKVKTTAPRRYCVRPNSGI 10 AP MASHEQALILEPAGELRFKGPFTDVVTADLKLSNPTDRRICFKVKTTAPKRYCVRPNSGI HP IDPGSTVTVSVMLQPFDYDPNEKSKHKFMVQTIFAPPNTSD-MEAVWKEAKPDELMDSKL AP LEPKTSIAVAVMLQPFNYDPNEKNKHKFMVQSMYAPDHVVESQELLWKDAPPESLMDTKL HP RCVFEMPNENDKLNDMEPSK-----AVPLNASKQDGPMPKP-HSVSLNDTE 15 AP RCVFEMPDGSHQAPASDASRATDAGAHFSESALEDPTVASRKTETQSPKRVGAVGSAGED HP TRKLMEECKRLOGEMMKLSEENRHLRDEGLRLRKVAHSD--KPGSTSTASFRDNVTSPLP AP VKKLOHELKKAOSEITSLKGENSOLKDEGIRLRKVAMTDTVSPTPLNPSPAPAAAVRAFP 20 HP SLLVVIAAIFIGFFLGKFIL ... \*.\*\*\*..\*..\*\*\* AP PVVYVVAAIILGLIIGKFLL

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA447905) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10423> (SEQ ID Nos. 4, 14, and 24)

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Determination of the whole base sequence of the cDNA insert of clone HP10423 obtained from cDNA library of human osteosarcoma cell line U-2 OS revealed the structure consisting of a 64-bp 5'-untranslated region, a 795-bp ORF, and a 207-bp 3'-untranslated region. The ORF codes for a protein consisting of 264 amino acid residues and there existed a secretory signal at the N-terminus and one putative transmembrane domain at the N-terminus. Figure 4 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 30 kDa that was almost identical with the molecular weight of 29,377 predicted from the ORF. When expressed in COS7 cells, an expression product of about 31 kDa was observed in the membrane fraction.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. D80116) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10506> (SEQ ID Nos. 5, 15, and 25)

Determination of the whole base sequence of the cDNA insert of clone HP10506 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 53-bp 5'-untranslated region, a 339-bp ORF, and a 226-bp 3'-untranslated region. The ORF codes for a protein consisting of 112 amino acid residues and there existed one putative transmembrane domain. Figure 5 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-

Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 12 kDa that was almost identical with the molecular weight of 11,821 predicted from the ORF. When expressed in COS7 cells, an expression product of about 13 kDa was observed in the membrane fraction.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA282544) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# 15 <HP10507> (SEQ ID Nos. 6, 16, and 26)

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Determination of the whole base sequence of the cDNA insert of clone HP10507 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 412-bp 5'-untranslated region, a 441-bp ORF, and a 168-bp 3'untranslated region. The ORF codes for a protein consisting of 146 amino acid residues and there existed a secretory signal at the N-terminus and one putative transmembrane at C-terminus. Figure 6 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. translation resulted in formation of a translation product of 19 kDa that was somewhat larger than the molecular weight of 16,347 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA424759) in ESTs, but, since they

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are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# 5 <HP10548> (SEQ ID Nos. 7, 17, and 27)

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Determination of the whole base sequence of the cDNA insert of clone HP10548 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 330-bp 5'-untranslated region, a 1035-bp ORF, and a 67-bp 3'untranslated region. The ORF codes for a protein consisting of 344 amino acid residues and there existed four putative Figure 7 depicts transmembrane domains. the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, the present protein. of translation resulted in formation of a translation product of a high molecular weight.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA143152) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP10566> (SEQ ID Nos. 8, 18, and 28)

Determination of the whole base sequence of the cDNA insert of clone HP10566 obtained from cDNA library of the human stomach cancer revealed the structure consisting of a 61-bp 5'-untranslated region, a 294-bp ORF, and a 246-bp 3'-untranslated region. The ORF codes for a protein consisting of 97 amino acid residues and there existed one putative transmembrane domain at the C-terminus. Figure 8 depicts the

hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 12 kDa that was almost identical with the molecular weight of 11,452 predicted from the ORF. When expressed in COS7 cells, an expression product of about 12 kDa was observed in the membrane fraction.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. W79821) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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# <HP10567> (SEQ ID Nos. 9, 19, and 29)

Determination of the whole base sequence of the cDNA insert of clone HP10567 obtained from cDNA library of the human stomach cancer revealed the structure consisting of a 77-bp 5'-untranslated region, a 375-bp ORF, and a 133-bp 3'-untranslated region. The ORF codes for a protein consisting of 124 amino acid residues and there existed one putative transmembrane domain at the C-terminus. Figure 9 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 14 kDa that was almost identical with the molecular weight of 14,484 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA428475) in ESTs, but, since they

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are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10568> (SEQ ID Nos. 10, 20, and 30)

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Determination of the whole base sequence of the cDNA insert of clone HP10568 obtained from cDNA library of the human stomach cancer revealed the structure consisting of a 56-bp 5'-untranslated region, a 984-bp ORF, and a 60-bp 3'untranslated region. The ORF codes for a protein consisting of 327 amino acid residues and there existed a secretory signal at the N-terminus and one putative transmembrane the C-terminus. Figure 10 depicts domain at hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. translation resulted in formation of a translation product of 36.5 kDa that was almost identical with the molecular weight of 34,326 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 40 kDa which is considered to have a sugar chain being In addition, there exist in the amino acid attached. sequence of this protein two sites at which N-glycosylation may occur (Asn-Leu-Thr at position 138 and Asn-Leu-Ser at position 206). Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from valine at position 24. When expressed in COS7 cells, an expression product of about 31 kDa was observed in the supernatant fraction and the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein has revealed that the protein was similar to the human cell-surface A33 antigen

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(SWISS-PROT Accession No. Q99795). Table 5 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human cell-surface A33 antigen (A3). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 30.0% in the N-terminal region of 243 residues.

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#### Table 5

HP MAELPGPFLCGALLGFLCLSGLAVEVKVPTEPLSTPLGKTAELTCTYSTSVGDSFAL-EW \*..\*..\* . \*... \*\*...\*.\*\*\* \*\*.... .\* .\*  ${\tt MVGKMWPVLWTLCAVRVTVDAISVETPQDVLRASQGKSVTLPCTYHTSTSSREGLIQW}$ 15 **A3** HP SFVOPGKPISESHPILYFTNGHLYPTGSKSKRVSLLQNPPTVGVATLKLTDVHPSDTGTY . .\*. \* \*.\* . \* \*. \*. A3 DKLL--LTHTERVVIWPFSNKN-YIHGELYKNRVSISNNAEQSDASITIDQLTMADNGTY HP LCQVNNPPDFYTNGLGLINLTVLVPPSNPLCSQSGQTSVGGSTALRCSSSEGAPKPVYNW 20 A3 ECSVSLMSDLEGNTKSRVRLLVLVPPSKPECGIEGETIIGNNIOLTCOSKEGSPTPOYSW HP VRLGTFPTPSPGSMVQDEVSGQLILTNLSLTSSGTYRCVATNQMGSASCELTLSVTEPS-A3 KRYNILNOEOP--LAOPASGOPVSLKNISTDTSGYYICTSSNEEGTQFCNITVAVRSPSM 25 HP -OGRVAGALIGVLLGVLLLSVAAFCLVRFQKERGKKPKETYGGSDLREDAIAPGISEHTC .\*\*. ...... .\* A3 NVALYVGIAVGVVAALIIIGIIIYCCCCRGKDDNTEDKEDARPNREAYEEPPEQLRELSR HP MRADSSKGFLERPSSASTVTTTKSKLPMVV 30 A3 EREEEDDYRQEEQRSTGRESPDHLDQ

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration

of sequences that shared a homology of 90% or more (for example, Accession No. T24595) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP01426> (SEQ ID Nos. 31, 41, and 51)

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Determination of the whole base sequence of the cDNA insert of clone HP01426 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 1-bp 5'-untranslated region, a 942-bp ORF, and a 122-bp 3'untranslated region. The ORF codes for a protein consisting of 313 amino acid residues and there existed a putative 11 depicts Figure signal. secretory hydrophobicity/hydrophilicity profile, obtained by the Kyteprotein. the present method. of translation resulted in formation of a translation product of 36 kDa that was almost identical with the molecular weight of 34,955 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 38 kDa which is considered to have a sugar chain being attached after secretion. In addition, there exists in the amino acid sequence of this protein one site at which Nglycosylation may occur (Asn-Ser-Ser at position 163). Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from tryptophan at position 17. When expressed in COS7 cells, an expression product of about 39 kDa was observed in the supernatant fraction and the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the

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protein was similar to the Xenopus laevis cortical granule lectin (EMBL Accession No. X82626). Table 6 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the X. laevis cortical granule lectin (XL). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 67.9% in the region other than the N-terminal region.

#### Table 6

HP MNQLSFLLFLIATTRGWSTDEANTYFKEWTCSSSPSLPRSCKEIKDECPSAFDGLYFLRT \*\*\*\*\*\*\* . \* \*\*.\* \* . 15 XL MLVHILLLLVTGGLSQSCEPVVIVASKNMVKQLDCDKFRSCKEIKDSNEEAQDGIYTLTS HP ENGVIYOTFCDMTSGGGGWTLVASVHENDMRGKCTVGDRWSSQQGSKADYPEGDGNWANY ..\*. \*\*\*\*\*\*\*\*..\*\*\*\*\*\*\*\*\*\*\*\*\*\*\*\* XL SDGISYQTFCDMTTNGGGWTLVASVHENNMAGKCTIGDRWSSQQGNRADYPEGDGNWANY 20 HP NTFGSAEAATSDDYKNPGYYDIOAKDLGIWHVPNKSPMQHWRNSSLLRYRTDTGFLQTLG \*\*\*\*\*\* . \*\*\*\*\*\*\*\*\*\* . \* . \*\*. \*\*\*\*\* . \* . \*\*\*\*\* \*\*\*\*..\*. \* XL NTFGSAGGATSDDYKNPGYYDIEAYNLGVWHVPNKTPLSVWRNSSLQRYRTTDGILFKHG HP HNLFGIYQKYPVKYGEGKCWTDNGPVIPVVYDFGDAQKTASYYSPYGQREFTAGFVOFRV 25 XL GNLFSLYRIYPVKYGIGSCSKDSGPTVPVVYDLGSAKLTASFYSPDFRSQFTPGYIOFRP HP FNNERAANALCAGMRVTGCNTEHHCIGGGGYFPEASPQQCGDFSGFDWSGYGTHVGYSSS \*\*\*\*\*\*\* XL INTEKAALALCPGMKMESCNVEHVCIGGGGYFPEADPRQCGDFAAYDFNGYGTKKFNSAG HP REITEAAVLLFYR 30 \*\*\*\*\* XL IEITEAAVLLFYL

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. R06009) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP02515> (SEQ ID Nos. 32, 42, and 52)

Determination of the whole base sequence of the cDNA insert of clone HP02515 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 176-bp 5'-untranslated region, a 690-bp ORF, and a 71-bp 3'-untranslated region. The ORF codes for a protein consisting of 229 amino acid residues and there existed a putative secretory signal at N-terminus and one putative transmembrane domain at the C-terminus. Figure 12 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 27 kDa that was almost identical with the molecular weight of 26,000 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 25.5 kDa from which the secretory signal is considered to have been cleaved. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from phenylalanine at position 28.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human T1/ST2 receptor binding protein (GenBank Accession No. U41804). Table 7 shows the

comparison between amino acid sequences of the human protein of the present invention (HP) and the human T1/ST2 receptor binding protein (T1). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 55.8% in the entire region.

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Table 7

T1 AFEARDRNLQEGNLERVNFWSAVNVAVLLLVAVLQVCTLKRFFQDKRPVPT

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA381943) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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WO 00/05367 PCT/JP99/03929

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<HP02575> (SEQ ID Nos. 33, 43, and 53)

Determination of the whole base sequence of the cDNA insert of clone HP02575 obtained from cDNA library of human line Saos-2 revealed osteosarcome cell the consisting of a 55-bp 5'-untranslated region, a 1404-bp ORF, and a 219-bp 3'-untranslated region. The ORF codes for a protein consisting of 467 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 13 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 52 kDa that was almost identical with the molecular weight of 54,065 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 57 kDa which is considered to have a sugar chain being attached afetr secretion. In addition, there exist in the amino acid sequence of this protein three sites at which N-qlycosylation may occur (Asn-Arg-Thr at position 171, Asn-Ser-Thr at position 239 and Asn-Asp-Thr at position 377). Application of the (-3,-1)rule, a method predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from histidine at position 29. When expressed in COS7 cells, an expression product of about 55 kDa was observed in the supernatant fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human  $\alpha$ -L-fucosidase (SWISS-PROT Accession No. P04066). Table 8 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human  $\alpha$ -L-fucosidase (FC). Therein,

the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 54.8% in the entire region.

# Table 8

	HP	MRPQELPRLAFPLLLLLLLPPPPC-PAHSATRFDPTWESLDARQLPAWFDQAKFGIFI
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	FC	MRSRPAGPALLLLLLFLGAAESVRRAQPPRRYTPDWPSLDSRPLPAWFDEAKFGVFI
	HP	HWGVFSVPSFGSEWFWWYWQKEKIPKYVEFMKDNYPPSFKYEDFGPLFTAKFFNANQWAD
		******* ****** * * * * * * * * * * * * *
	FC	${\tt HWGVFSVPAWGSEWFWWHWQGEGRPQYQRFMRDNYPPGFSYADFGPQFTARFFHPEEWAD}$
15	HP	IFQASGAKYIVLTSKHHEGFTLWGSEYSWNWNAIDEGPKRDIVKELEVAIRNRTDLRFGL
		.***.***.***.***
	FC	LFQAAGAKYVVLTTKHHEGFTNWPSPVSWNWNSKDVGPHRDLVGELGTALRKR-NIRYGL
	HP	YYSLFEWFHPLFLEDESSSFHKRQFPVSKTLPELYELVNNYQPEVLWSDGDGGAPDQYWN
		*.**.***** ** .**.********* .**.***
20	FC	YHSLLEWFHPLYLLDKKNGFKTQHFVSAKTMPELYDLVNSYKPDLIWSDGEWECPDTYWN
	HP	STGFLAWLYNESPVRGTVVTNDRWGAGSICKHGGFYTCSDRYNPGHLLPHKWENCMTIDK
		**.**.**.** * *** *
	FC	STNFLSWLYNDSPVKDEVVVNDRWGQNCSCHHGGYYNCEDKFKPQSLPDHKWEMCTSIDK
	HP	${\tt LSWGYRREAGISDYLTIEELVKQLVETVSCGGNLLMNIGPTLDGTISVVFEERLRQMGSWarder}$
25		.**********.** *** *.**** **
	FC	${\tt FSWGYRRDMALSDVTEESEIISELVQTVSLGGNYLLNIGPTKDGLIVPIFQERLLaVGKW}$
	HP	${\tt LKVNGEAIYETHTWRSQNDTVTPDVWYTSKPKEKLVYAIFLKWPTSGQLFLGHPKAILGA}$
		******** * ****** ******.**.
	FC	${\tt LSINGEAIYASKPWRVQWEKNTTSVWYTSKGSAVYAIFLHWPENGVLNLESPITT-ST}$
30	HP	TEVKLLGHGQPLNWISLEQNGIMVELPQLTIHQMPCKWGWALALTNVI
		*** *.*******
	FC	TKITMLGIQGDLKWSTDPDKGLFISLPQLPPSAVPAEFAWTIKLTGVK

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. N28668) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10357> (SEQ ID Nos. 34, 44, and 54)

Determination of the whole base sequence of the cDNA insert of clone HP10357 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 113-bp 5'-untranslated region, a 300-bp ORF, and a 54-bp 3'untranslated region. The ORF codes for a protein consisting of 99 amino acid residues and there existed two putative transmembrare domains. Figure 14 depicts hydrophobicity/hydrophilicity profile, obtained by the Kyteof Doolittle method, the present protein. In translation resulted in formation of a translation product of 11 kDa that was almost identical with the molecular weight of 10,923 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA477156) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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<HP10447> (SEQ ID Nos. 35, 45, and 55)

Determination of the whole base sequence of the cDNA

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insert of clone HP10447 obtained from cDNA library of human liver revealed the structure consisting of a 271-bp 5'untranslated region, a 570-bp ORF, and a 34-bp untranslated region. The ORF codes for a protein consisting of 189 amino acid residues and there existed five putative domains. Figure 15 depicts transmembrare hydrophobicity/hydrophilicity profile, obtained by the Kyteof Doolittle method, the present protein. translation resulted in formation of a translation product of high molecular weight.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA296976) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP10477> (SEQ ID Nos. 36, 46, and 56)

Determination of the whole base sequence of the cDNA insert of clone HP10477 obtained from cDNA library of human liver revealed the structure consisting of a 149-bp 5'-untranslated region, a 1092-bp ORF, and a 15-bp 3'-untranslated region. The ORF codes for a protein consisting of 363 amino acid residues and there existed one putative transmembrane domain at the N-terminus. Figure 16 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 40 kDa that was almost identical with the molecular weight of 39,884 predicted from the ORF.

The search of the protein data base using the amino

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acid sequence of the present protein revealed that the protein was similar to the human peptidoglycan recognition protein (GenBank Accession No. AF076483). Table 9 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human peptidoglycan recognition protein (PG). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 54.8% in the entire region.

#### Table 9

HP MVDSLLAVTLAGNLGLTFLRGSQTQSHPDLGTEGCWDQLSAPRTFTLLDPKASLLTKAFL
HP NGALDGVILGDYLSRTPEPRPSLSHLLSQYYGAGVARDPGFRSNFRRQNGAALTSASILA
HP QQVWGTLVLLQRLEPVHLQLQCMSQEQLAQVAANATKEFTEAFLGCPAIHPRCRWGAAPY

\*..\* \*\* \* \* ...

PG SECAQHLSLPLRYVVVSHT--AGSSCNTPASCQQQARNVQHYHMKTLGWCDVGYNFLIGE

HP DGYVYEGRGWHWVGAHTLGH-NSRGFGVAIVGNYTAALPTEAALRTVRDTLPSCAVRAGL

PG DGLVYEGRGWNFTGAHSGHLWNPMSIGISFMGNYMDRVPTPQAIRAAQGLL-ACGVAQGA

HP LRPDYALLGHRQLVRTDCPGDALFDLLRTWPHFTATVKPRPARSVSKRSRREPPPRTLPA

\*\*..\*.\* \*\*\*.. \*\* .\*\*..\*..\*..\*\*

PG LRSNYVLKGHRDVQRTLSPGNQLYHLIQNWPHYRSP

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration

of sequences that shared a homology of 90% or more (for example, Accession No. AA424759) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10513> (SEQ ID Nos. 37, 47, and 57)

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Determination of the whole base sequence of the cDNA insert of clone HP10513 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 134-bp 5'-untranslated region, a 750-bp ORF, and a 0-bp 3'-untranslated region. The ORF codes for a protein consisting of 249 amino acid residues and there existed one putative transmembrane domain at the N-terminus. Figure 17 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 29 kDa that was almost identical with the molecular weight of 27,373 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human hypothetical protein KIAA0512 (GenBank Accession No. AB011084). Table 10 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human hypothetical protein KIAA0512 (KI). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 31.6% in the C-terminal region of 196 amino acid residues.

### Table 10

HP MGGPRGAGWVAAGLLLGAGACYCIYRLTRGRRRG 5 KI RGRGRRPVAMOKRPFPYEIDEILGVRDLRKVLALLQKSDDPFIQQVALLTLSNNANYSCN HP DRELGIRSSKSAEDLTDGSYDDVLNAEQLQKLLYLLESTEDPVIIERALITLGNNAAFSV \* ....\* . \* \*. \*.. .. . .. .. KI OETIRKLGGLPIIANMINKTDPHIKEKALMAMNNLSENYENQGRLQVYMNKVMDDIMASN 10 HP NOAIIRELGGIPIVANKINHSNOSIKEKALNALNNLSVNVENQIKIKVQVLKLLLNLSEN ... .\*... \* . ..... ... ... KI LNSAVQVVGLKFLTNMTITNDYQHLLVNSIANF--FRLLSQGGGKIKVEILKILSNFAEN HP PAMTEGLLRAQVDSSFLSLYDSHVAKEILLRVLTLFQNIKNCLKIEGHLAVQPTFTEGSL 15 KI PDMLKKLLSTOVPASFSSLYNSYVESEILINALTLFEIIYDNLRAE--VFNYREFNKGSL HP FFL-LHGEECAOKIRALVDHHDAEVKEKVVTIIPKI . \* \*\*\*\*\* \*\* \*\* \*\* \*\* \*\* KI FYLCTTSGVCVKKIRALANHHDLLVKVKVIKLVNKF

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. N92228) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10540> (SEQ ID Nos. 38, 48, and 58)

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Determination of the whole base sequence of the cDNA insert of clone HP10540 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure

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consisting of a 47-bp 5'-untranslated region, a 297-bp ORF, and a 245-bp 3'-untranslated region. The ORF codes for a protein consisting of 98 amino acid residues and there existed two putative transmembrane domains. Figure 18 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein revealed that the similar the Caenorhabditis protein was to hypothetical protein CEF49C12.12 (GenBank Accession Z68227). Table 11 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein CEF49C12.12 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 36.1% in the entire region.

#### Table 11

<sup>25</sup> HP M-ASLLCCGPKLAACGIVLSAWGVIMLIMLGIFFNVHSAVLIEDVPFTEKDFENGPQNIY

CE MGKICPLMGPKMSAFCMVMSVWGVIFLGLLGVFFYIQAVTLFPDLHF-EGHGKVPSSVID HP NLYEQVSYNCFIAAGLYLLLGGFSFCQVRLNKRKEYMVR

<sup>\* \* \*\*\*\*\* \* \* \*\*</sup> 

<sup>30</sup> CE AKYNEKATQCWIAAGLYAVTLIAVFWQ---NKYNTAQIF

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA420715) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# 10 <HP10557> (SEQ ID Nos. 39, 49, and 59)

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Determination of the whole base sequence of the cDNA insert of clone HP10557 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 24-bp 5'-untranslated region, a 519-bp ORF, and a 130-bp 3'untranslated region. The ORF codes for a protein consisting of 172 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 19 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. translation resulted in formation of a translation product of 32 kDa that was larger than the molecular weight of 18,844 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 39 kDa is considered to have been subjected to some which modification after secretion. In addition, there exist in the amino acid sequence of this protein no site at which Nglycosylation may occur. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from glycine at position 32. When expressed in COS7 cells, an expression product of about 20 kDa was observed in the supernatant fraction and the membrane fraction.

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The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human progesterone binding protein (EMBL Accession No. AJ002030). Table 12 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human progesterone binding protein (PG). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 30.5% in the C-terminal region of 151 amino acid residues.

#### Table 12

15 HP **MVGPAP** PG MAAGDGDVKLGTLGSGSESSNDGGSESPGDAGAAAEGGGWAAAALALLTGGGEMLLNVAL HP RRRLRPLAALALVLALAPGLPTARAGQTPRPAERGPPV--RLFTEEELARYGGEEEDOPI 20 PG VALVLLGAYRLWVRWGRRGLGAGAGAGEESPATSLPRMKKRDFSLEQLRQYDG-SRNPRI HP YLAVKGVVFDVTSGKEFYGRGAPYNALTGKDSTRGVAKMSLDPADLTHDTTGLTAKELEA \*\*\*.\* \*\*\*\*\*.\*..\*\*\*...\*\*...\*\*...\*\* ..\* ... ... PG LLAVNGKVFDVTKGSKFYGPAGPYGIFAGRDASRGLATFCLDKDALRDEYDDLSDLNAVQ 25 HP LDEV--FTKVYKAKYPIVGYTARRILNEDGSPNLDFKPEDQPHFDIKDEF ...\* ... .\*.\*\* .\*.. \*.\*. ...\*. ... \*.... . \*.. PG MESVREWEMQFKEKY---DYVG-RLLKPGEEPS-EYTDEEDTKDHNKQD

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for

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example, Accession No. AA101709) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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## <HP10563> (SEQ ID Nos. 40, 50, and 60)

Determination of the whole base sequence of the cDNA insert of clone HP10563 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 126-bp 5'-untranslated region, a 363-bp ORF. and a 936-bp 3'-untranslated region. The ORF codes for a protein consisting of 120 amino acid residues and there existed two putative transmembrane domains. depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 18.5 kDa that was larger than the molecular weight of 13,180 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Arabidopsis thaliana hypothetical protein F27F23.15 (GenBank Accession No. AC003058). Table 13 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the A. thaliana hypothetical protein F27F23.15 (AT). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 35.5% in the entire region.

#### Table 13

HP MMPSRTNLATGIPSSKVKYSRLSSTDDGYIDLQFKKTPPKIPYKAIALATVLFLIGAFLI \*..\* \*. . . . . \* \*.\*. \* \* . . . \* MAYVDHAFSISDEDLMIGTSY-TVSNRPPVKEISLAVGLLVFGTLGI AT HP IIGSLLLSGYISKGGADRAVPVLIIGILVFLPGFYHLRIAYYASKGYRGYSYDDIPDFDD ..\* .. . .. \*. .... ...\* \*.\*.\*\*\* \*\*\*\*\* \*\*\*.\*.\*\* AT VLGFFMAYNRVG-GDRGHGIFFIVLGCLLFIPGFYYTRIAYYAYKGYKGFSFSNIPSV

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA083574) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP01467> (SEQ ID Nos. 61, 71, and 81)

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Determination of the whole base sequence of the cDNA insert of clone HP01467 obtained from cDNA library of human fibrosarcoma cell line HT-1080 revealed the structure consisting of a 65-bp 5'-untranslated region, a 924-bp ORF, and a 447-bp 3'-untranslated region. The ORF codes for a protein consisting of 307 amino acid residues and there existed three putative transmembrane domains. Figure 21 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

The search of the protein data base using the amino

acid sequence of the present protein revealed that the protein was similar to the rat Sec22 homologue (GenBank Accession No. U42209). Table 14 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the rat Sec22 homologue (RN). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 94.6% in the N-terminal region of 241 amino acid residues. The protein of the present invention was longer by 53 amino acids at the C-terminus than the rat Sec22 homologue.

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#### Table 14

HP MSMILSASVIRVRDGLPLSASTDYEQSTGMQECRKYFKMLSRKLAQLPDRCTLKTGHYNI \* RN MSMILSASVVRVRDGLPLSASTDCEQSAGVQECRKYFKMLSRKLAOFPDRCTLKTGRHNI 20 HP NFISSLGVSYMMLCTENYPNVLAFSFLDELQKEFITTYNMMKTNTAVRPYCFIEFDNFIQ \*\*\*\*\*\*\*\*\*\*\*\* RN NFISSLGVSYMMLCTENYPNVLAFSFLDELQKEFITTYNMMKTNTAVRPYCFIEFDNFIO HP RTKQRYNNPRSLSTKINLSDMQTEIKLRPPYQISMCELGSANGVTSAFSVDCKGAGKISS \*\*\*\*\*\*\*\*\*\*\*\* 25 RN RTKQRYNNPRSLSTKINLSDMQMEIKLRPPYQIPMCELGSANGVTSAFSVDCKGAGKISS HP AHQRLEPATLSGIVGFILSLLCGALNLIRGFHAIESLLQSDGDDFNYIIAFFLGTAACLY \*\*\*\*\*\*\*\*\*\* RN AHQRLEPATLSGIVAFILSLLCGALNLIRGFHAIESLLQSDGEDFSYMIAFFLGTAACLY HP QCYLLVYYTGWRNVKSFLTFGLICLCNMYLYELRNLWQLFFHVTVGAFVTLQIWLRQAQG 30

RN QMICLCLQGRKERT

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA421925) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP01956> (SEQ ID Nos. 62, 72, and 82)

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Determination of the whole base sequence of the cDNA insert of clone HP01956 obtained from cDNA library of human liver revealed the structure consisting of a 86-bp 5'untranslated region, a 552-bp ORF, and a 359-bp 3'untranslated region. The ORF codes for a protein consisting of 183 amino acid residues and there existed one putative 22 depicts transmembrane domain. Figure hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. translation resulted in formation of a translation product of 20.5 kDa that was almost identical with the molecular weight of 20,073 predicted from the ORF.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the yeast hypothetical protein 21.5 kDa (SWISS-PROT Accession No. P53073). Table 15 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the yeast hypothetical protein 21.5 kDa (SC). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology

of 34.3% in the C-terminal region of 108 amino acid residues.

### Table 15

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA159753) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02545> (SEQ ID Nos. 63, 73, and 83)

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Determination of the whole base sequence of the cDNA insert of clone HP02545 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 133-bp 5'-untranslated region, a 984-bp ORF, and a 636-bp 3'-untranslated region. The ORF codes for a

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protein consisting of 327 amino acid residues and there existed a putative secretory signal at the N-terminus and one putative transmembrane domain at the C-terminus. Figure 23 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein.

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The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the rat embigin (EMBL Accession No. AJ009698). Table 16 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the rat embigin (RN). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 65.4% in the entire region.

## Table 16

HP MRALPGLLEARARTPRLLLLQCLLAAARPSSADGSAPDSPFTSPPLREEIMAN--NFSLE 5 RN MRSHTGLRALVAPGCSLLLL-YLLAATRPDRAVGDPADSAFTSLPVREEMMAKYANLSLE HP SHNISLTEHSSMPVEKNITLERPSNVNLTCQFTTSGDLNAVNVTWKKDGEQLE--NNYLV ..\*\*\*\*\*.... \*.\*\*\*\*\*\*\*...\*. ..\*. ..\*\* RN TYNISLTEQTRVS-EQNITLERPSHLELECTFTATEDVMSMNVTWKKDDALLETTDGFNT HP SATGSTLYTQYRFTIINSKQMGSYSCFFREEKEQRGTFNFKVPELHGKNKPLISYVGDST 10 . \*.\*\*\*.\*\*\*\*..\*\*\*\*..\*\* \*\*\*\*\* . . \* \* . . \* \* \* \* \* \* \* . \* \* \* \* \* \* RN TKMGDTLYSQYRFTVFNSKQMGKYSCFLGEE--LRGTFNIRVPKVHGKNKPLITYVGDST HP VLTCKCQNCFPLNWTWYSSNGSVKVPVGVQM-NKYVINGTYANETKLKITOLLEEDGESY \*\*\*\*\*\*\*\*\*\*\*\* RN VLKCECQNCLPLNWTWYMSNGTAQVPIDVHVNDKFDINGSYANETKLKVKHLLEEDGGSY 15 HP WCRALFQLGESEEHIELVVLSYLVPLKPFLVIVAEVILLVATILLCEKYTOKKKKHSDEG RN WCRAAFPLGESEEHIKLVVLSFMVPLKPFLAIIAEVILLVAIILLCEVYTOKKKNDPDDG HP KEFEQIEQLKSDDSNGIENNVPRHRKNESLGQ \*\*\*\*\*\*\*\*\*\*\*\*\*\*\* 20 RN KEFEQIEQLKSDDSNGIENNVPRYRKTDSGDQ

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the reqistration of sequences that shared a homology of 90% or more (for example, Accession No. AA312629) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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<HP02551> (SEQ ID Nos. 64, 74, and 84)

Determination of the whole base sequence of the cDNA insert of clone HP02551 obtained from cDNA library of human

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line Saos-2 revealed the structure osteosarcoma cell consisting of a 61-bp 5'-untranslated region, a 672-bp ORF, and a 384-bp 3'-untranslated region. The ORF codes for a protein consisting of 223 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 24 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 27 kDa that was somewhat larger than the molecular weight of 24,555 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 26 kDa from which the secretory signal is considered to have been cleaved. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from glutamine at position 20.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the mouse FGF binding protein (GenBank Accession No. U49641). Table 17 comparison between amino acid sequences of the human protein of the present invention (HP) and the mouse FGF binding protein (MM). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 21.2% in the entire region other than the N-terminal region. In particular, all the eight cysteine residues contained in the both proteins were conserved.

## Table 17

MKFVPCLLLVTLSCLGTLGQAPRQKQGST HP ..\*\*. . .\* . ... 5 MM MRLHSLILLSFLLLATQAFSEKVRKRAKNAPHSTAEEGVEGSAPSLGKAQNKORSRTSKS HP GEEFHFQTGGRDSCTMRPSSLGQGAGEVWLRVDCRNTDQTYWCEYRGQPSMCOAFAADPK .. .\* \* ....\* . ..... .. \*.\*.\* ..\*\*.. \* . \*.\*. \* MM LTHGKFVTKDQATC---RWAVTEEEQGISLKVQCTQADQEFSCVFAGDPTDCLKHDKD-O HP SYWNQALQELRRLHHACQGA-PVLRPSVCREAGPQAHMQQVTSSLKGSPEPNOOPEAGTP 10 MM IYWKQVARTLRKQKNICRDAKSVLKTRVCRKRFPESNLKLVNPNARGNTKPRKEKAEVSA HP SLRPKATVKLTEATQLGKDSMEELGKAKPTTRPTAKPTQPGPRPGGNEEAKKKAWEHCWK ..... \*... .\*. \* . \*. \* . ... MM REHNKVQEAVSTEPNRIKEDI-TLNPAATQTM-TIRDPECLEDPDVLNQ-RKTALEFCGE 15 HP PFQALCAFLISFFRG MM SWSSICTFFLNMLQATSC

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA317400) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP02631> (SEQ ID Nos. 65, 75, and 85)

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Determination of the whole base sequence of the cDNA insert of clone HP02631 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 42-bp 5'-untranslated region, a 147-bp ORF,

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and a 1191-bp 3'-untranslated region. The ORF codes for a protein consisting of 48 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 25 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 10 kDa or less.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA156969) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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# <HP02632> (SEQ ID Nos. 66, 76, and 86)

Determination of the whole base sequence of the cDNA insert of clone HP02632 obtained from cDNA library of human fibrosarcoma cell line HT-1080 revealed the structure consisting of a 50-bp 5'-untranslated region, a 1116-bp ORF, and a 337-bp 3'-untranslated region. The ORF codes for a protein consisting of 371 amino acid residues and there existed eight putative transmembrane domains. Figure 26 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Caenorhabditis elegans hypothetical protein CELC2H12 (GenBank Accession No. U23169). Table 18 shows the comparison between amino acid sequences

of the human protein of the present invention (HP) and the C. elegans hypothetical protein CELC2H12 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 51.4% in the entire region.

## Table 18

10 HP MAWTKYQLFLAGLMLVTGSINTLSAKWADNFMAEGCGGSKEHSFQHPFLQAVGMFLGEFS MVAFAVIISVMMVVTGSLNTICAKWADSIKAD-----GVPFNHPFLQATCMFFGEFL CE HP CLAAFYL-----LRCRAAGQSDS-----SVDPQQPFNPLLFLPPALCDMTGTSL 15 \* ...\*.\*.\* . . \*\*\*\* \*\* \*\*\*\*\* \*\*\* CE CLVVFFLIFGYKRYVWNRANVQGESGSVTEITSEEKPTLPPFNPFLFFPPALCDILGTSI HP MYVALNMTSASSFQMLRGAVIIFTGLFSVAFLGRRLVLSQWLGILATIAGLVVVGLADLL \*\*..\*\*.\*.\*.\*.\*\*\*\*\*\*\*\*\*\*\* CE MYIGLNLTTASSFQMLRGAVIIFTGLLSVGMLNAQIKPFKWFGMLFVMLGLVIVGVTDIY 20 HP SKHDSQHKLSEVITGDLLIIMAQIIVAIQMVLEEKFVYKHNVHPLRAVGTEGLFGFVILS CE YDDDPLDDKNAIITGNLLIVMAQIIVAIQMVYEQKYLTKYDVPALFAVGLEGLFGMVTLS HP LLLVPMYYIPAG-SFSGNPRGTLEDALDAFCQVGQQPLIAVALLGNISSIAFFNFAGISV 25 CE ILMIPFYYIHVPRTFSTNPEGRLEDVFYAWKEITEEPTIALALSGTVVSIAFFNFAGVSV HP TKELSATTRMVLDSLRTVVIWALSLALGWEAFHALQILGFLILLIGTALYNGLHRPLLGR CE TKELSATTRMVLDSVRTLVIWVVSIPLFHEKFIAIQLSGFAMLILGTLIYNDILIGPWFR HP LSRGRPLAEESEQERLLGGTRTPINDAS 30 CE RNILPNLSSHANCARCWLCICGGDSELIEYEQEDQEHLMEA

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. N50907) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP10488> (SEQ ID Nos. 67, 77, and 87)

Determination of the whole base sequence of the cDNA insert of clone HP10488 obtained from cDNA library of human liver revealed the structure consisting of a 39-bp 5'-untranslated region, a 273-bp ORF, and a 421-bp 3'-untranslated region. The ORF codes for a protein consisting of 90 amino acid residues and there existed one putative transmembrane domain at the N-terminus. Figure 27 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 10 kDa that was almost identical with the molecular weight of 10,151 predicted from the ORF. When expressed in COS7 cells, an expression product of about 6 kDa was observed in the membrane fraction.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. H73534) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

Determination of the whole base sequence of the cDNA insert of clone HP10538 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 357-bp 5'-untranslated region, a 1500-bp ORF, and a 1911-bp 3'-untranslated region. The ORF codes for a protein consisting of 499 amino acid residues and there existed at least four putative transmembrane domains. Figure 28 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the mouse pore-forming K<sup>+</sup> channel subunit (GenBank Accession No. AF056492). Table 19 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the mouse pore-forming K<sup>+</sup> channel subunit (MM). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 32.4% in the N-terminal region of 241 amino acid residues.

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## Table 19

HP MVDRGPLLTSAIIFYLAIGAAIFEVLEEPHWKEAKKNYYTQKLHLLKEFPCLGOEGLDK \* . ...\*\*. \*\* .\*..\*\* ..\*.\*. . ..\*.. \*\*..\*..\*. 5 MM MRSTTLLALLALVLLYLVSGALVFQALEQPHEQQAQKKMDHGRDQFLRDHPCVSOKSLED HP ILEVVSDAAGOG----VAITGNOTFNNWNWPNAMIFAATVITTIGYGNVAPKTPAGRLF ..... .\* \* \* .... ..\*\* .\*..\*.\*\*\*\*\*\*\* . . . \* \*\*\*\*\* MM FIKLLVEALGGGANPETSWTNSSNHSSAWNLGSAFFFSGTIITTIGYGNIVLHTDAGRLF HP CVFYGLFGVPLCLTWISALGKFFGGRAKR----LGQFLTKRGVSLRKAQITCTVIFIVWG 10 \*.\*\*.\* \*.\*\* ....\*. .\*. .\* .... \*. \*. MM CIFYALVGIPLFGMLLAGVGDRLGSSLRRGIGHIEAIFLKWHVPPGLVRSLSAVLFLLIG HP VLVHLVIPPFVFMVTEGWNYIEGLYYSFITISTIGFGDFVAGVNPSANYHALYRYFVEI.W \*. ...\*.\*\* \*.\*. .\*..\*. ..\*.\*.\*.\* . ... . \*. .\*. MM CLLFVLTPTFVFSYMESWSKLEAIYFVIVTLTTVGFGDYVPG-DGTGQNSPAYQPLVWFW 15 HP IYLGLAWLSLFVNWKVSMFVEVHKAIKKRRRRKESFESSPHSRKALQVKGSTASKDVNI \* .\*\*\*... MM ILFGLAYFASVLTTIGNWLRAVSRRTRAEMGGLTAQAASWTGTVTARVTQRTGPSAPPPE

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. R25184) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10542> (SEQ ID Nos. 69, 79, and 89)

Determination of the whole base sequence of the cDNA insert of clone HP10542 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 23-bp 5'-untranslated region, a 321-bp ORF, and a 426-bp 3'-

WO 00/05367

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PCT/JP99/03929

untranslated region. The ORF codes for a protein consisting of 106 amino acid residues and there existed one putative transmembrane domain. Figure 29 depicts hydrophobicity/hydrophilicity profile, obtained by the Kytemethod, of the present protein. Doolittle translation resulted in formation of a translation product of 12 kDa that was almost identical with the molecular weight of 11,724 predicted from the ORF. When expressed in COS7 cells, an expression product of about 13 kDa was observed in the membrane fraction.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA029683) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10571> (SEQ ID Nos. 70, 80, and 90)

Determination of the whole base sequence of the cDNA insert of clone HP10571 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 95-bp 5'-untranslated region, a 459-bp ORF, and a 675-bp 3'untranslated region. The ORF codes for a protein consisting of 152 amino acid residues and there existed one putative transmembrane 30 domain. Figure depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, the present protein. of In vitro translation resulted in formation of a translation product of 20 kDa that was larger than the molecular weight of 17,062 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 23 kDa

PCT/JP99/03929

which is considered to have a sugar chain being attached after secretion. In addition, there exists in the amino acid sequence of this protein one site at which N-glycosylation may occur (Asn-Ile-Thr at position 10).

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA105822) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP01470> (SEQ ID Nos. 91, 101, and 111)

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Determination of the whole base sequence of the CDNA insert of clone HP01470 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 157-bp 5'-untranslated region, a 1077-bp ORF, and a 385-bp 3'untranslated region. The ORF codes for a protein consisting of 358 amino acid residues and there existed one putative 31 transmembrane domain. Figure depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of present the protein. translation resulted in formation of a translation product of 43 kDa that was somewhat larger than the molecular weight of 40,489 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 40 kDa from which the secretory signal is considered to have been cleaved and a product of 43.5 kDa which is considered to have been subjected to some modification. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from glycine at position 23. When

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expressed in COS7 cells, an expression product of about 44 kDa was observed in the supernatant fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the to the was similar Caenorhabditis protein hypothetical protein 39.9 kDa (SWISS-PROT Accession No. Q10005). Table 20 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein 39.9 kDa (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 58.9% in the entire region.

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#### Table 20

HP MAPQNLSTFCLLLLYLIGAVIAGRDFYKILGVPRSASIKDIKKAYRKLALQLHPDRNPDD \*. \* \*\*\*\*\*\*\*\*\* ... .. .. \*\*\*\*\*\*\* . \*\*\*\*\* 5 CE MRILNVSLLVLASSLVAFVECGRDFYKILGVAKNANANQIKKAYRKLAKELHPDRNODD HP PQAQEKFQDLGAAYEVLSDSEKRKQYDTYGEEGL--KDGHQSSHGDIFSHFFGDFGFMFG \*.\*\*\*\*\* ... \*\*\*\*\* \*\* .\*\*\* ... CE EMANEKFQDLSSAYEVLSDKEKRAMYDRHGEEGVAKMGGGGGGGHDPFSSFFGDF-FG-G HP GTPRQQDRNIPRGSDIIVDLEVTLEEVYAGNFVEVVRNKPVARQAPGKRKCNCROEMRTT 10 CE GGGHGGEEGTPKGADVTIDLFVTLEEVYNGHFVEIKRKKAVYKQTSGTRQCNCRHEMRTE HP QLGPGRFQMTQEVVCDECPNVKLVNEERTLEVEIEPGVRDGMEYPFIGEGEPHVDGEPGD CE OMGOGRFOMFOVKVCDECPNVKLVOENKVLEVEVGADNGHOOIFHGEGEPHIEGDPGD 15 HP LRFRIKVVKHPIFERRGDDLYTNVTISLVESLVGFEMDITHLDGHKVHISRDKITRPGAK CE LKFKIRIQKHPRFERKGDDLYTNVTISLQDALNGFEMEIQHLDGHIVKVORDKVTWPGAR HP LWKKGEGLPNFDNNNIKGSLIITFDVDFPKEQLTEEAREGIKQLLKQGSVO-KVYNGLOG \*.\*\*.\*\*.\*....\*\* \*\* \*...\*\*\*...\*...\*... \* ...\*...\*.. \* ...\*... 20 CE LRKKDEGMPSLEDNNKKGMLVVTFDVEFPKTELSDEQKAQIIEILQONTVKPKAYNGL

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA282838) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

30 <HP002419> (SEQ ID Nos. 92, 102, and 112)

Determination of the whole base sequence of the cDNA insert of clone HP02419 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 253-bp

5'-untranslated region, a 681-bp ORF, and a 1120-bp 3'-untranslated region. The ORF codes for a protein consisting of 226 amino acid residues and there existed four putative transmembrane domains. Figure 32 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human hypothetical protein KIAA0108 (SWISS-PROT Accession No. Q15012). Table 21 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human hypothetical protein KIAA0108 (KI). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 43.9% in the entire region.

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## Table 21

ΗP MKMVAPWTRFYSNSCCLCCHVRTGTILLGVWYLIINAVVLLILLSALADPD---OY \*\*\*\*..\* \*\*\*\*\*\*\*\*.\*\*...\* ...\* KI MVSMSFKRNRSDRFYSTRCCGCCHVRTGTIILGTWYMVVNLLMAILLTVEVTHPNSMPAV 5 HP NFSSELGGDFEF-MDDANMCIAIAISLLMILICAMATYGAYKQRAAWIIPFFCYQIFDF KI NIOYEVIGNYYSSERMADNACVLFAVSVLMFIISSMLVYGAISYQVGWLIPFFCYRLFDF HP ALNMLVAITVLIYPNSIQEYIRQLPPNFPYRDDVMSVNPTCLVLIILLFISIILTFKGYL 10 KI VLSCLVAISSLTYLPRIKEYLDQL-PDFPYKDDLLALDSSCLLFIVLVFFALFIIFKAYL HP ISCVWNCYRYINGRNSSDVLVYVT-SNDTTVLLPPYDDATVNGAAKEPPPPYVSA KI INCVWNCYKYINNRNVPEIAVYPAFEAPPQYVLPTY-EMAVKMPEKEPPPPYLPA 15

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA173214) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP02631> (SEQ ID Nos. 93, 103, and 113)

Determination of the whole base sequence of the cDNA insert of clone HP02631 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 42-bp 5'-untranslated region, a 588-bp ORF, and a 750-bp 3'-untranslated region. Although the 49th amino acid residue is encoded by a stop codon, it is likely that this codon encodes selenocysteine from the molecular weight

WO 00/05367

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PCT/JP99/03929

of the translation product and the sequence comparison data with the Caenorhabditis elegans homologue. The ORF codes for a protein consisting of 195 amino acid residues and there existed a putative secretory signal at the N-terminus and one putative transmembrane domain in the intermediate region. Figure 33 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 58 kDa. In this case, the addition of a microsome led to the formation of a product of 56 kDa from which the secretory signal is considered to have been cleaved. Since both of these products are larger than the molecular weight of 22 kDa predicted from the ORF, it is likely that the protein interacts with another protein.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Caenorhabditis elegans hypothetical protein C35C5.3 (EMBL Accession No. 278417). Table 22 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein C35C5.3 (CE). U at position 49 in the amino acid sequence of the protein of the present invention represents selenocysteine. Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 37.9% in the entire region other than the Nterminal region. Cystein was found in the sequence of the C. elegans protein at the posistion corresponding to position 49 encoded by the stop codon (selenocysteine) of the protein of the present invention.

### Table 22

HP MRLLLL 5 CE MRIHDELQKQDMSRFGVFIIGVLFFMSVCDVLRTEEHSHDENHVHEKDDFEAEFGDETDS HP LLVAASAMVRSEASANLGGVPSKRLKMQYATGPLLKFQICVSUGYRRVFEEYMRVISQRY CE OSFSOGTEEDHIEVREOSSFVKPTAVHAKDLPTLRIFYCVSCGYKQAFDOFTTFAKEKY HP PDIRIEGENYLPQPIYRHIASFLSVFKLVLIGLIIVGKDPFAFFGMQAPSIWQWGQENKV 10 ... \*\* \*... \*.. \* .\*\*. \*\*. \* \* \* ... \*\*. \*...\*\*\*.\*. \* CE PNMPIEGANFAPVLWKAYVAQALSFVKMAVLVLVLGGINPFERFGLGYPQILQHAHGNKM HP YACMMVFFLSNMIENQCMSTGAFEITLNDVPVWSKLESGHLPSMQQLVQILDNEMKLNVH CE SSCMLVFMLGNLVEQSLISTGAFEVYLGNEQIWSKIESGRVPSPQEFMQLIDAQLAVLGK 15 HP MDSIPHHRS CE APVNTESFGEFOOTV

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA156969) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP02695> (SEQ ID Nos. 94, 104, and 114)

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Determination of the whole base sequence of the cDNA insert of clone HP02695 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 112-bp 5'-untranslated region, a 1020-bp ORF, and a 160-bp 3'-

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untranslated region. The ORF codes for a protein consisting of 339 amino acid residues and there existed three putative transmembrane domains. Figure 34 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kytemethod, of the present protein. translation resulted in formation of a translation product of 38 kDa that was almost identical with the molecular weight of 38,274 kDa predicted from the ORF.

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The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the rat hypertension-induced protein S-2 fragment (PIR Accession No. 539959). Table 23 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the rat hypertension-induced protein S-2 fragment (RN). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 74.3% in the entire region.

## Table 23

HP MNWELLLWLLVLCALLLLLVOLLRFLRADGDLTLLWAEWQGRRPEWELTDMVVWVTGASS

HP GIGEELAYQLSKLGVSLVLSARRVHELERVKRRCLENGNLKEKDILVLPLDLTDTGSHEA

RN VKRRSLENGNLKEKDILVLPLDLADTSSHDI

HP ATKAVLQEFGRIDILVNNGGMSQRSLCMDTSLDVYRKLIELNYLGTVSLTKCVLPHMIER

RN ATKTVLQEFGRIDILVNNGGVAHASLVENTNMDIFKVLIEVNYLGTVSLTKCFLPHMMER

HP KQGKIVTVNSILGIISVPLSIGYCASKHALRGFFNGLRTELATYPGIIVSNICPGPVQSN

RN NQGKIVVMKS

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. T84331) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10031> (SEQ ID Nos. 95, 105, and 115)

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Determination of the whole base sequence of the cDNA insert of clone HP10031 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 55-bp 5'-untranslated region, a 1464-bp ORF, and a 649-bp 3'-untranslated region. The ORF codes for a protein consisting of 487 amino acid residues and there existed eleven putative transmembrane domains. Figure 35 depicts the hydrophobicity/hydrophilicity profile, obtained

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by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight. When expressed in COS7 cells, an expression product of about 55 kDa was observed in the membrane fraction.

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The search of the protein data base using the amino acid sequence of the present protein revealed that the the Caenorhabditis was similar to protein hypothetical protein CELK07H8 (GenBank Accession AF047659). Table 24 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein CELK07H8 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 44.2% in the entire region.

### Table 24

MDGTETRQRRLDSCGKPGELGLPHPLSTGGLPVAS HP 5 CE MKGGGGIGDGKKDYQSAVHEGLTTFDQLGIALEDVGKSMDAETATPGGSLFSRVIFRFRN HP EDGALRAPESQSVTPKPLETEPSRETAWSIGLQVTVPFMFAGLGLSWAGMLLDYFQHWPV . . \*... . .\*\* \*\* \*\*\*\*. .\*\*..\*. CE ENSSLKSRTYDHSNDLVNMSVIPAESSYVLFFQVLFPFAVAGLGMVFAGLVLSIVVTWPL HP FVEVKDLLTLVPPLVGLKGNLEMTLASRLSTAANTGQIDDPQEQHRVISSNLALIQVQAT 10 CE FEEIPEILILVPALLGLKGNLEMTLASRLSTLANLGHMDSSKQRKDVVIANLALVQVQAT HP VVGLLAAVAALLLGVVSREEVDVAKVELLCASSVLTAFLAAFALGVLMVCIVIGARKLGV CE VVAFLASAFAAALAFIPSGDFDWAHGALMCASSLATACSASLVLSLLMVVVIVTSRKYNI 15 HP NPDNIATPIAASLGDLITLSILALVSSFFYR-HKDSRYLTPLVCLSFAALTPVWVLIAKO \* CE NPDNVATPIAASLGDLTTLTVLAFFGSVFLKAHNTESWLNVIVIVLFLLLLPFWIKIANE HP SPPIVKILKFGWFPIILAMVISSFGGLILSKTVSKOOYKGMAIFTPVICGVGGNLVAIOT 20 CE NEGTOETLYNGWTPVIMSMLISSAGGFILETAV--RRYHSLSTYGPVLNGVGGNLAAVOA HP SRISTYLHMWSAPGVLPLO--MKKFWPNPCSTFCTSEINSMSARVLLLLVVPGHLIF-FY CE SRLSTYFHKAGTVGVLPNEWTVSRF-TSVORAFFSKEWDSRSARVLLLLVVPGHICFNFL HP I-IYLVEGOSVINSO--TFVVLYLLAGLIOVTILLYLAEVMVRLTWHOALDPDNHCIPYL 25 CE IQLFTLTSKNNVTPHGPLFTSLYMIAAIIQVVILLFVCQLLVALLWKWKIDPDNSVIPYL HP TGLGDLLGTGLLALCFFTDWLLKSKAELGGISELASGPP \*.\*\*\*\*\*\*\* CE TALGDLLGTGLLFIVFLTTDHFDPKELTSS 30

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for

94

example, Accession No. AA334000) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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# <HP10530> (SEQ ID Nos. 96, 106, and 116)

Determination of the whole base sequence of the cDNA insert of clone HP10530 obtained from cDNA library of human Saos-2 revealed osteosarcoma cell line the structure consisting of a 80-bp 5'-untranslated region, a 1182-bp ORF, and a 95-bp 3'-untranslated region. The ORF codes for a protein consisting of 393 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 36 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 46 kDa that was somewhat larger than the molecular weight of 44,912 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 45.5 kDa from which the secretory signal is considered to have been cleaved. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from lysine at position 23. When expressed in COS7 cells, an expression product of about 43 kDa was observed in the supernatant fraction and the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Arabidopsis thaliana hypothetical protein IG002N01 (GenBank Accession No. AF007269). Table 25 shows the comparison between amino acid sequences of the

human protein of the present invention (HP) and the A. thaliana hypothetical protein IG002N01 (AT). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 27.0% in the N-terminal region of 355 amino acid residues.

## Table 25

HP MRTLFNLLWL 5 AT MELTSFQKSPSSNDVVSFSVSLVRNSMARRRRSSAAESLKRRNDGYESLCQVVQQDSDRR HP ALACSPVHTTLSKSDAKKAASKTLLEKSQFSDKPVQDRGLVVTDLKAESVVLEHRSYCSA AT LITIFVIFFIVIPAVSIAVYKVKFADRVIQTESSIRQKGIVKTDINFQEILTEHSK--AS HP KARDRHFAGDVLGYVTPWNSHGYDVTKVFGSKFTQISPVWLQ-LKRRGREMFEVTGLHDV 10 AT ENSTRHYDYPVLAYITP--CQGSGL--VLEGR-HNADKGWIQELRSRGNALSASKGLPKL HP DOGWMRAVRKHAKGLHIVPRLLFEDWTYDDFRNVLDSEDEIEELSKTVVQVAKNQHFDGF . . . . \* . . . \* . \*\* . . . \* . \* . . . . . . . . AT ---YNSCIFHALKRMNFFTLELVNFNTYLVIMFALNS-REMEYNGIVLESWSRWAAYGVL 15 HP VVEVWNQLLSQKRVGLIHMLTHLAEALHQARLLALLVIPPAITPGTDQLGMFTHKEFEQL \* . \* ... ... \*. \*. \*. \*... \* AT HDPDLRKMALKFVKQLGDALHSTSSPRNNQQHMQFMYVVGPPRSEKLQMYDFGPEDLQFL HP APVLDGFSLMTYDYSTAHQPGPNAPLSWVRACVQ-VLDPKSK----WRSKILLGLNFYGM \*\*\*\*\*\*\* 20 AT KDSVDGFSLMTYDFSNPQNPGPNAPVKWIDLTLKLLLGSSNNIDSNIARKVLLGINFYGN HP DYATSKDAREPVVGARYIQTLKDHRPRMVWDSQASEHFFEYKKSRSGRHVVFYPTLKSLQ \*...\* .. ....\* \*.. \*..\* . \*\*....\*\*.\* \*.... . . \*.\*\*\*\*\*.\*. AT DFVISGGGGGAITGRDYLALLOKHKPTFRWDKESGEHLFMYRDDKNIKHAVFYPTLMSIL HP VRLELARELGVGVSIWELGQGLDYFYDLL 25 AT LRLENARLWGIGISIWEIGODKGHFGKYAEASLEASSIFSGHTFDMQFRTNPRQLSRNGS

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA302913) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the

WO 00/05367

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protein of the present invention.

<HP10541> (SEQ ID Nos. 97, 107, and 117)

Determination of the whole base sequence of the cDNA insert of clone HP10541 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 7-bp 5'-untranslated region, a 591-bp ORF, and a 113-bp 3'untranslated region. The ORF codes for a protein consisting of 196 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 37 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kytemethod, of the present protein. Doolittle translation resulted in formation of a translation product of 23 kDa that was somewhat larger than the molecular weight of 21,553 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of 20 kDa from which the secretory signal is considered to have been cleaved and a product of 23 kDa which is considered to have a sugar chain being attached. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from glycine at position 41. In addition, there exists in the amino acid sequence of this protein one site at which N-glycosylation may occur (Asn-Leu-Thr at position 185).

PCT/JP99/03929

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human zymogen membrane protein (GenBank Accession No. AF056492). Table 26 comparison between amino acid sequences of the human protein of the present invention (HP) and the human zymogen membrane protein (ZM). Therein, the marks of -, \*, and . represent a

98

gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 37.6% in the C-terminal region of 133 amino acid residues.

### Table 26

HP MWRVPGTTRRPVTGESPGMHRPEAMLLLLTLALLGGPTWAGKMYGPGGGKYFS-TTEDYD 10 \*\*.\*\*\* \*\* . . . \* MLTVALLALLCASASGNAIQARSSSYSGEYGSGGGKRFSHSGNOLD ZM HP HEITGLRVSVGLLLVKSVQVKLGDSWDVKLGALGGNTQEVTLQPGEYITKVFVAFQAFLR ZM GPITALRVRVNTYYIVGLQVRYGKVWSDYVGGRNGDLEEIFLHPGESVIQVSGKYKWYLK 15 HP GMVMYTSKDRYFYFGKLDGQISSAYPSQEGQVLVGIYGQYQLLGIKSIGFEWN-YPLEEP .\*. \*.\*.\*\*. \*\*\* .\* . . \*\* \* \*. \* \*..\*\*..\*. \*\* ZM KLVFVTDKGRYLSFGKDSGTSFNAVPLHPNTVLRFISGRSGSL-IDAIGLHWDVYPTSCS HP TTEPPVNLTYSANSPVGR 20 ZM RC

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA340605) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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<HP10550> (SEQ ID Nos. 98, 108, and 118)
Determination of the whole base sequence of the cDNA

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insert of clone HP10550 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 241-bp 5'-untranslated region, a 324-bp ORF, and a 86-bp 3'-untranslated region. The ORF codes for a protein consisting of 107 amino acid residues and there existed one putative transmembrane domain. Figure 38 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA348310) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10590> (SEQ ID Nos. 99, 109, and 119)

Determination of the whole base sequence of the cDNA insert of clone HP10590 obtained from cDNA library of human fibrosarcoma cell line HT-1080 revealed the structure consisting of a 77-bp 5'-untranslated region, a 1053-bp ORF, and a 180-bp 3'-untranslated region. The ORF codes for a protein consisting of 350 amino acid residues and there existed one putative transmembrane domain. Figure 39 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 40 kDa that was almost identical with the molecular weight of 39,285 predicted from the ORF. In this case, the addition of a microsome led to the formation of a product of

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43 kDa which is considered to have a sugar chain being attached. In addition, there exist in the amino acid sequence of this protein two sites at which N-glycosylation may occur (Asn-Asn-Ser at position 144 and Asn-Leu-Thr at position 328).

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA461346) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10591> (SEQ ID Nos. 100, 110, and 120)

Determination of the whole base sequence of the cDNA insert of clone HP10591 obtained from cDNA library of human fibrosarcoma cell line HT-1080 revealed the structure consisting of a 232-bp 5'-untranslated region, a 324-bp ORF, and a 844-bp 3'-untranslated region. The ORF codes for a protein consisting of 107 amino acid residues and there existed one putative transmembrane domain. Figure 40 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 12 kDa that was almost identical with the molecular weight of 11,328 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. H09424) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein

of the present invention.

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<HP01462> (SEQ ID Nos. 121, 131, and 141)

Determination of the whole base sequence of the cDNA insert of clone HP01462 obtained from cDNA library of human line HT-1080 revealed the structure fibrosarcoma cell consisting of a 121-bp 5'-untranslated region, a 1452-bp ORF, and a 477-bp 3'-untranslated region. The ORF codes for a protein consisting of 483 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 41 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 72 kDa that was larger than the 55,838 predicted molecular weight of from the Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from lysine at position 21.

The search of the protein data base using the amino acid sequence of the present protein revealed that the to the Caenorhabditis similar was hypothetical protein ZK1058.4 (EMBL Accession No. Z35604). Table 27 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein ZK1058.4 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 35.6% in the entire region.

## Table 27

HP MKAFHTFCVVLLVFGSVSEAKFDDFEDEEDIVEYDDNDFAEFEDVMEDSVTESPORVIIT 5 CE MKIVWIFLIFFIGFAIST HP EDDE-DETTVELEGODENOEGDFEDADTQEGDTESEPYDDEEFEGYEDKP-----D .\*.\* .\* . \*. \* ...\*. ... .... ...\*.\*. CE DDNEFAEFEDEFVGSSATOAPEIOREGEPPVLKQKDDFEEEDFGVVEEEPEEAEKVREAD HP TSSSKNKDPITIVDVPAHLONSWESYYLEILMVTGLLAYIMNYIIGKNKNSRLAQAWFNT 10 CE SDDAAPAQPLKFADVPAHFRSNWASYQVEGIVVLIILIYMTNYLIGKTTNASIAQTIFDM HP HRELLESNFTLVGDDGTNKEATSTGKLNQENEHIYNLWCSGRVCCEGMLIQLRFLKRQDL \* \*\*..\*..\*\*\*\*\* CE CRPTLEEQFAVVGDDGTTDLDKMIPSLKHDTDSTFSAWCTGRVNVNSLFLQMKMVKRQDV HP LNVLARMMRPVSDQVQIKVTMN-DEDMDTYVFAVGTRKALVRLQKEMQDLSEFCSDKPKS 15 .. . \* . \* . \* . . . . . . \* \* . . \* \* \* . . \* CE VSRIMEMFTPSGDKMTIKASLETTNDTDPLIFAVGEKKIASKYFKEMLDLNSFASERKOA HP GAKYGLPDSLAILSEMGEVTDGMMDTKMVHFLTHYADKIESVHFSDQFSGPKIMQEEGQP 20 CE AOOFNLPASWOVYADONEVVFSILDPGVVSLLKKHEDAIEFIHISDQFTGPKPAEGESYT HP LKLPDTKRTLLFTFNVPGSGNTYPKDMEALLPLMNMVIYSIDKAKKFRLNREGKQKADKN .\*\*... \* .\* \*... ..\*.\* \*\*\*\*.\*..\* ... \* \*\*... CE -RLPEAORYMFVSLNLOYLG----QDEESVMEILNLVFYLIDKARKMKLSKDAKVKAERR HP RARVEENFLKLTHVOROEAAOSRREEKKRAEKERIMNEEDPEKQRRLEEAALRREQKKLE 25 CE RKEFEDAFLKQTHQFRQEAAQARREEKTRERKQKLMDESDPERQKRLEAKELKREAKA--HP KKOMKMKOIKVKAM \* \*\*\*\* \*\*\* CE -KSPKMKOLKVK 30

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for

103

example, Accession No. AA307793) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

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<HP02485> (SEQ ID Nos. 122, 132, and 142)

Determination of the whole base sequence of the cDNA insert of clone HP02485 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 69-bp 5'-untranslated region, a 1005-bp ORF, and a 1672-bp 3'untranslated region. The ORF codes for a protein consisting of 334 amino acid residues and there existed one putative transmembrane domain. Figure 42 depicts hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. translation resulted in formation of a translation product of 36 kDa that was almost identical with the molecular weight of 38,171 predicted from the ORF. When expressed in COS7 cells, an expression product of about 23 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the similar to the Caenorhabditis was hypothetical protein W01A11.2 (GenBank Accession No. U64852). Table 28 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein W01A11.2 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 45.5% in the entire region.

### Table 28

HP MVEFAPLFMPWERRLQTLAVLQFVFSFLALAEICT-V .\*\*\*..\*\*.\*\*\*\*\* \*.\* \*. \*. 5 CE MRLRLSSISGKAKLPDKEICSSVSRILAPLLVPWKRRLETLAVMGFIFMWVILPIMDLWV HP GFIALLFTRFWLLTVLYAAWWYLDRDKPROGGRHIQAIRCWTIWKYMKDYFPISLVKTAE \* .\*. \*\*.\*. \*\*\*.\* \* \*.\*...\* . \* . \*\*\*. .\*\*..\*. CE PFHVLFNTRWWFLVPLYAVWFYYDFDTPKKASRRWNWARRHVAWKYFASYFPLRLIKTAD 10 HP LDPSRNYIAGFHPHGVLAVGAFANLCTESTGFSSIFPGIRPHLMMLTLWFRAPFFRDYIM \* ..\*\*\* \* \*\*\*\*...\*\*.\*...\*.. \*\*\*\*.. \* \* \* \* \* \* . . . CE LPADRNYIIGSHPHGMFSVGGFTAMSTNATGFEDKFPGIKSHIMTLNGQFYFPFRREFGI HP SAGLVTSEKESAAHILNRKGGGNLLGIIVGGAQEALDARPGSFTLLLRNRKGFVRLALTH \* .. .\*\*\* ...\*. \* \*. .\*.\*\*\* \*\*\*.\*.\*. \*\* \* \*\*.\*\* . \*\*. 15 CE MLGGIEVSKESLEYTLTKCGKGRACAIVIGGASEALEAHPNKNTLTLINRRGFCKYALKF HP GAPLVPIFSFGENDLFDQIPNSSGSWLRYIQNRLQKIMGISLPLFHGRGVF-QYSFGLIP CE GADLVPMYNFGENDLYEOYENPKGSRLREVQEKIKDMFGLCPPLLRGRSLFNQYLIGLLP HP YRRPITTVVGKPIEVOKTLHPSEEEVNOLHQRYIKELCNLFEAHKLKFNIPADQHLEFC .\*.\*.\*\*.\*.\* \* .\* .\*. \*....\* ..\* .\*\*\*\*\*..\* 20 CE FRKPVTTVMGRPIRVTQTDEPTVEQIDELHAKYCDALYNLFEEYKHLHSIPPDTHLIFQ

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. D25664) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP02798> (SEQ ID Nos. 123, 133, and 143)

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Determination of the whole base sequence of the cDNA

105

insert of clone HP02798 obtained from cDNA library of human fibrosarcoma cell line HT-1080 revealed the structure consisting of a 31-bp 5'-untranslated region, a 804-bp ORF, and a 301-bp 3'-untranslated region. The ORF codes for a protein consisting of 267 amino acid residues and there existed four putative transmembrane domains. Figure 43 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 29 kDa that was almost identical with the molecular weight of 30,778 predicted from the ORF. When expressed in COS7 cells, an expression product of about 26 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the human DHHC-containing cysteinerich protein (GenBank Accession No. U90653). Table 29 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human DHHCcontaining cysteine-rich protein (DH). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 35.0% in the intermediate region of 100 amino The positions of seven cysteines were acid residues. conserved between the two proteins. The protein of the present invention also had the DHHC (Asp-His-His-Cys) sequence.

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PCT/JP99/03929

WO 00/05367

106

## Table 29

HP MAPWALLSPGVLVRTGHTVLTWGI 5 DH MYKMNICNKPSNKTAPEKSVWTAPAQPSGPSPELQGQRSRRNGWSWPPHPLQIVAWLLYL HP TLVLFLHDTELRQWEEQGELLLPLTFLLLVLGSLLLYLAVSLMDPGYVNVQPQP-QEELK \* \*...\*.. .\*\*. .\*\*. . . . \* DH FFAVIGFGILVPLLPHHWVPAGYACMGAIFAGHLVVHLTAVSIDPADDNVRDKSYAGPLP HP EEQTAMVPPAIPLRRCRYCLVLQPLRARHCRECRRCVRRYDHHCPWMENCVGERNHPLFV 10 .\*. \* \* . \*..\*\*..\*\* .\*\*\* \*..\*\*\*\*\*\*..\*\*. DH IFNRSOHAHVIEDLHCNLCNVDVSARSKHCSACNKCVCGFDHHCKWLNNCVGERNYRLFL HP VYLALQLVVLLWGLYLAWSGLRFFQPWGLWLRSSGLLFATFLLLSLFSLVASLLLVSHLY .\* .\*. .\* DH HSVASALLGVLLLVLGGHICLRGVLCQPHASAHQPTL 15

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. D79050) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10041> (SEQ ID Nos. 124, 134, and 144)

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Determination of the whole base sequence of the cDNA insert of clone HP10041 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 12-bp 5'-untranslated region, a 321-bp ORF, and a 286-bp 3'-untranslated region. The ORF codes for a protein consisting of 106 amino acid residues and there existed one putative transmembrane domain. Figure 44 depicts

the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 12 kDa that was almost identical with the molecular weight of 12,060 predicted from the ORF. When expressed in COS7 cells, an expression product of about 13 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the similar to the Caenorhabditis protein was elegans hypothetical protein K10B2.4 (GenBank Accession No. U28730). Table 30 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the C. elegans hypothetical protein K10B2.4 (CE). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The both proteins shared a homology of 62.1% in the entire region.

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## Table 30

HP MSTNNMSDPRRPNKVLRYKP---PPSECNPALDDPTPDYMNLLGMIFSMCGLMLKLKWCA

CE MQQNGDPRTTNRIVRYKPLDSTANQQQAISEDPLPEYMNVLGMIFSMCGLMIRMKWCS

HP WVAVYCSFISFANSRSSEDTKOMMSSFMLSISAVVMSYLQNPQPMTPPW

CE WLALVCSCISFANTRTSDDAKQIVSSFMLSVSAVVMSYLQNPSPIIPPWVTLLQS

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Furthermore, the search of the GenBank using the base

PCT/JP99/03929

sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. H20098) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10246> (SEQ ID Nos. 125, 135, and 145)

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Determination of the whole base sequence of the cDNA insert of clone HP10246 obtained from cDNA library of human epidermoid carcinoma cell line KB revealed the structure consisting of a 110-bp 5'-untranslated region, a 675-bp ORF, and a 79-bp 3'-untranslated region. The ORF codes for a protein consisting of 224 amino acid residues and there existed five putative transmembrane domains. Figure 45 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 23 kDa that was somewhat smaller than the molecular weight of 25,244 predicted from the ORF. When expressed in COS7 cells, an expression product of about 21 kDa was observed in the membrane fraction.

The search of the protein data base using the amino acid sequence of the present protein revealed that the similar protein was to the human putative transmembrane domain protein (GenBank Accession No. Y18007). Table 31 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and the human putative seven transmembrane domain protein (TM). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that

PCT/JP99/03929

109

of the protein of the present invention, respectively. The both proteins shared a homology of 93.3% in the entire region.

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WO 00/05367

### Table 31

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Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA453931) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

<HP10392> (SEQ ID Nos. 126, 136, and 146)

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Determination of the whole base sequence of the cDNA insert of clone HP10392 obtained from cDNA library of human osteosarcoma cell line U-2 OS revealed the structure

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consisting of a 24-bp 5'-untranslated region, a 777-bp ORF, and a 726-bp 3'-untranslated region. The ORF codes for a protein consisting of 258 amino acid residues and there existed a putative secretory signal at the N-terminus. Figure 46 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 34 kDa that was somewhat larger than the molecular weight of 29,623 predicted from the ORF. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from leucine at position 49.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. H15999) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention. In addition, partial identity with the hypothetical protein KIAA0384 (Accession No. AB002382) was observed, although the hypothetical protein had a different ORF.

# <HP10489> (SEQ ID Nos. 127, 137, and 147)

Determination of the whole base sequence of the cDNA insert of clone HP10489 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 137-bp 5'-untranslated region, a 333-bp ORF, and a 189-bp 3'-untranslated region. The ORF codes for a protein consisting of 110 amino acid residues and there existed two putative transmembrane domains. Figure 47 depicts the

hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of 19 kDa that was somewhat larger than the molecular weight of 12,010 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA262162) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

## <HP10519> (SEQ ID Nos. 128, 138, and 148)

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Determination of the whole base sequence of the cDNA insert of clone HP10519 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 67-bp 5'-untranslated region, a 276-bp ORF, and a 367-bp 3'untranslated region. The ORF codes for a protein consisting of 91 amino acid residues and there existed one putative transmembrane domain. Figure 48 depicts hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, the present protein. of translation resulted in formation of a translation product of 10 kDa that was almost identical with the molecular weight of 10,275 predicted from the ORF.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. W16639) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein

of the present invention.

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<HP10531> (SEQ ID Nos. 129, 139, and 149)

Determination of the whole base sequence of the cDNA insert of clone HP10531 obtained from cDNA library of human osteosarcoma cell line Saos-2 revealed the structure consisting of a 55-bp 5'-untranslated region, a 1035-bp ORF, and a 1092-bp 3'-untranslated region. The ORF codes for a protein consisting of 344 amino acid residues and there existed five putative transmembrane domains. Figure 49 depicts the hydrophobicity/hydrophilicity profile, obtained by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight.

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. R50695) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

# <HP10574> (SEQ ID Nos. 130, 140, and 150)

Determination of the whole base sequence of the cDNA insert of clone HP10574 obtained from cDNA library of human stomach cancer revealed the structure consisting of a 210-bp 5'-untranslated region, a 1287-bp ORF, and a 1276-bp 3'-untranslated region. The ORF codes for a protein consisting of 428 amino acid residues and there existed a putative secretory signal at the N-terminus and one putative transmembrane domain in the intermediate region. Figure 50 depicts the hydrophobicity/hydrophilicity profile, obtained

113

by the Kyte-Doolittle method, of the present protein. In vitro translation resulted in formation of a translation product of high molecular weight. Application of the (-3,-1) rule, a method for predicting the cleavage site of the secretory signal sequence, allows to expect that the mature protein starts from serine at position 36.

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The search of the protein data base using the amino acid sequence of the present protein revealed that the protein was similar to the Drosophila melanogaster GOLIATH protein (SWISS-PROT Accession No. Q06003). Table 32 shows the comparison between amino acid sequences of the human protein of the present invention (HP) and melanogaster GOLIATH protein (DM). Therein, the marks of -, \*, and . represent a gap, an amino acid residue identical with that of the protein of the present invention, and an amino acid residue similar to that of the protein of the present invention, respectively. The intermediate region of 169 amino acids of the protein of the present invention shared a homology of 41.4% with the N-terminal region of the D. melanogaster GOLIATH protein.

#### Table 32

HP MGPPPGAGVSCRGGCGFSRLLAWCFLLALSPQAPGSRGAEAVWTAYLNVSWRVPHTGVNR HP TVWELSEGVYGODSPLEPVAGVLVPPDGPGALNACNPHTNFTVPTVWGSTVOVSWLALT 5 HP QRGGGCTFADKIHLAYERGASGAVIFNFPGTRNEVIPMSHPGAVDIVAIMIGNLKGTKIL DM MQLEKMQIKGKTRNIAAVITYONIGODLS HP OSIORGIOVTMVIEVGKK---HGPWVNHYSIFFVSVSFFIITAATVGYFIFYSARRLRNA ....\* .\*\*. \* \*.. . .\*. \*..\*\*\*.\* \*\*. .... ..\*\*\* .\*.\* 10 DM LTLDKGYNVTISIIEGRRGVRTISSLNRTSVLFVSIS-FIV-DDILCWLIFYYIQRFRYM HP RAQSRKQRQLKADAKKAIGRLQLRTLKQGDKEIGPDGDSCAVCIELYKPNDLVRILTCNH .\*... \*.\* . .\*\*\* ... .\* \* .\* . \* .\*.\*\* \*\*\* \*\*\*.\* .\*\*\*.\* DM QAKDQQSRNLCSVTKKAIMKIPTKTGKFSD-EKDLDSDCCAICIEAYKPTDTIRILPCKH HP IFHKTCVDPWLLEHRTCPMCKCDILKALGIEVDVEDGSVSLQVPVSNEISNSASSHEEDN 15 \*\*\*.\*.\*\*\*\*\*\*\*\* \*.\*\* \* \*. DM EFHKNCIDPWLIEHRTCPMCKLDVLKFYGYVVGDOIYOTPSPOHTAPIASIEEVPVIVVA HP RSETASSGYASVQGTDEPPLEEHVQSTNESLQLVNHEANSVAVDVIPHVDNPTFEEDETP DM VPHGPQPLQPLQASNMSSFAPSHYFQSSRSPSSSVQQQLAPLTYQPHPQQAASERGRRNS 20 HP NOETAVREIKS DM APATMPHAITASHQVTDV

Furthermore, the search of the GenBank using the base sequences of the present cDNA has revealed the registration of sequences that shared a homology of 90% or more (for example, Accession No. AA155685) in ESTs, but, since they are partial sequences, it can not be judged whether or not any of these sequences codes for the same protein as the protein of the present invention.

#### INDUSTRIAL APPLICABILITY

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The present invention provides human proteins having hydrophobic domains, DNAs coding for these proteins, and expression vectors for these DNAs as well as eucaryotic cells expressing these DNAs. All of the proteins of the present invention are secreted or exist in the cell membrane, so that they are considered to be proteins controlling the proliferation and/or the differentiation of the cells. Accordingly, the proteins of the present invention can be employed as pharmaceuticals such as carcinostatic agents which act to control the proliferation and/or the differentiation of the cells, or as antigens for preparing antibodies against these proteins. The DNAs of the present invention can be utilized as probes for the genetic diagnosis and gene sources for the gene therapy. Furthermore, the DNAs can be utilized for large-scale expression of these proteins. Cells into which these genes are introduced to express these proteins, can be utilized for detection of the corresponding receptors and ligands, screening of novel lowmolecular pharmaceuticals, and so on.

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herein.

to the polynucleotide sequences disclosed "Corresponding genes" are the regions of the genome that are transcribed to produce the mRNAs from which polynucleotide sequences are derived and may contiguous regions of the genome necessary for the regulated expression of such genes. Corresponding genes may therefore include but are not limited to coding sequences, 5' and 3' untranslated regions, alternatively spliced exons, introns, promoters, enhancers, and silencer or suppressor elements. The corresponding genes can be isolated in accordance with known methods using the sequence information disclosed

Such methods include the preparation of probes or

The present invention also provides genes corresponding

116

primers from the disclosed sequence information for identification and/or amplification of genes in appropriate genomic libraries or other sources of genomic materials. An "isolated gene" is a gene that has been separated from the adjacent coding sequences, if any, present in the genome of the organism from which the gene was isolated.

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Organisms that have enhanced, reduced, or modified qene(s) expression of the corresponding to the polynucleotide sequences disclosed herein are provided. The desired change in gene expression can be achieved through the use of antisense polynucleotides or ribozymes that bind and/or cleave the mRNA transcribed from the gene (Albert and Sci. 1994, Trends Pharmacol. 15(7): 250-254; Morris, Lavarosky et al., 1997, Biochem. Mol. Med. 62(1): 11-22; and Hampel, 1998, Proq. Nucleic Acid Res. Mol. Biol. 58: 1-39; of which are incorporated by reference herein). Transgenic animals that have multiple copies of the gene(s) corresponding to the polynucleotide sequences disclosed herein, preferably produced by transformation of cells with genetic constructs that are stably maintained within the transformed cells and their progeny, are provided. modified genetic Transgenic animals that have regions that increase or reduce gene expression levels, or that change temporal or spatial patterns of gene expression, are also provided (see European Patent No. 0 649 464 Bl, incorporated by reference herein). In addition, organisms are provided in which the gene(s) corresponding to the disclosed polynucleotide sequences herein have been partially or completely inactivated, through insertion of extraneous sequences into the corresponding gene(s) through deletion of all or part of the corresponding gene(s). Partial or complete gene inactivation can be accomplished

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117

through insertion, preferably followed by imprecise excision, of transposable elements (Plasterk, 1992, Bioessays 14(9): 629-633; Zwaal et al., 1993, Proc. Natl. Acad. Sci. USA 90(16): 7431-7435; Clark et al., 1994, Proc. Natl. Acad. Sci. 91(2): 719-722; all of which are incorporated by reference herein), or through homologous recombination, preferably detected by positive/negative genetic selection strategies (Mansour et al., 1988, Nature 336: 348-352; U.S. Patent Nos. 5,464,764; 5,487,992; 5,627,059; 5,631,153; 5,614, 396; 5,616,491; and 5,679,523; all of which are incorporated by reference herein). These organisms with altered gene expression are preferably eukaryotes and more Such organisms are useful for the preferably are mammals. development of non-human models for the study of disorders involving the corresponding gene(s), and for the development of assay systems for the identification of molecules that interact with the protein product(s) of the corresponding gene(s). Where the protein of the present invention is membrane-bound (e.g., is a receptor), the present invention also provides for soluble forms of such protein. forms part or all of the intracellular and transmembrane domains of the protein are deleted such that the protein is fully secreted from the cell in which it is expressed. intracellular and transmembrane domains of proteins of the invention can be identified in accordance with known techniques for determination of such domains from sequence information.

Proteins and protein fragments of the present invention include proteins with amino acid sequence lengths that are at least 25%(more preferably at least 50%, and most preferably at least 75%) of the length of a disclosed protein and have at least 60% sequence identity (more

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preferably, at least 75% identity; most preferably at least 90% or 95% identity) with that disclosed protein, where sequence identity is determined by comparing the amino acid sequences of the proteins when aligned so as to maximize overlap and identity while minimizing sequence gaps. Also included in the present invention are proteins and protein fragments that contain a segment preferably comprising 8 or more (more preferably 20 or more, most preferably 30 or more) contiguous amino acids that shares at least 75% sequence identity (more preferably, at least 85% identity; most preferably at least 95% identity) with any such segment of any of the disclosed proteins.

Species homologs of the disclosed polynucleotides and proteins are also provided by the present invention. As "species homologue" is a protein herein, а polynucleotide with a different species of origin from that of a given protein or polynucleotide, but with significant sequence similarity to the given protein or polynucleotide, as determined by those of skill in the art. Species homologs may be isolated and identified by making suitable probes or primers from the sequences provided herein and screening a suitable nucleic acid source from the desired species.

The invention also encompasses allelic variants of the disclosed polynucleotides or proteins; that is, naturally-occurring alternative forms of the isolated polynucleotide which also encode proteins which are identical, homologous, or related to that encoded by the polynucleotides.

The invention also includes polynucleotides with sequences complementary to those of the polynucleotides disclosed herein.

The present invention also includes polynucleotides

119

capable of hybridizing under reduced stringency conditions, more preferably stringent conditions, and most preferably highly stringent conditions, to polynucleotides described herein. Examples of stringency conditions are shown in the table 33 below: highly stringent conditions are those that are at least as stringent as, for example, conditions A-F; stringent conditions are at least as stringent as, for example, conditions G-L; and reduced stringency conditions are at least as stringent as, for example, conditions M-R.

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Table 33

Stringency	Polynucleotide	Hybrid	Hybridization Temperature	Wash
Condition	Hybrid	Length	and Buffer†	Temperature
		(bp) <sup>‡</sup>		and Buffer <sup>†</sup>
A	DNA : DNA	≥50	65°C; 1×SSC -or-	65°C; 0.3×SSC
			42°C; 1×SSC,50% formamide	
В	DNA : DNA	<50	T <sub>B</sub> *; 1×SSC	T <sub>B</sub> *; 1×SSC
C	DNA: RNA	≥50	67°C; 1×SSC -or-	67°C; 0.3×SSC
			45°C; 1×SSC,50% formamide	
D	DNA : RNA	<50	T <sub>D</sub> *; 1×SSC	Tp*; 1×SSC
E	RNA: RNA	≥50	70°C; 1×SSC -or-	70°C; 0.3×SSC
			50°C; 1×SSC,50% formamide	
F	RNA: RNA	<50	T <sub>F</sub> *; 1×SSC	T <sub>F</sub> *; 1×SSC
G	DNA : DNA	≥50	65°C; 4×SSC -or-	65°C; 1×SSC
			42°C; 4×SSC,50% formamide	
Н	DNA : DNA	<50	T <sub>H</sub> *; 4×SSC	T <sub>H</sub> *; 4×SSC
I	DNA : RNA	≥50	67°C; 4×SSC -or-	67℃; 1×SSC
			45°C; 4×SSC,50% formamide	
J	DNA : RNA	<50	T <sub>J</sub> *; 4×SSC	T <sub>J</sub> *; 4×SSC
K	RNA: RNA	≥50	70°C; 4×SSC -or-	67°C; 1×SSC
ļ			50℃; 4×SSC,50% formamide	
L	RNA: RNA	<50	T <sub>L</sub> *; 2×SSC	T <sub>L</sub> *; 2×SSC
M	DNA : DNA	≥50	50°C; 4×SSC -or-	50°C; 2×SSC
			40°C; 6×SSC,50% formamide	
N	DNA : DNA	<50	T <sub>N</sub> *; 6×SSC	T <sub>N</sub> *; 6×SSC
0	DNA: RNA	≥50	55°C; 4×SSC -or-	55°C; 2×SSC
			42℃; 6×SSC,50% formamide	
P	DNA: RNA	<50	T <sub>P</sub> *; 6×SSC	T <sub>P</sub> *; 6×SSC
Q	RNA: RNA	≥50	60°C; 4×SSC -or-	60°C; 2×SSC
	·		45°C; 6×SSC,50% formamide	
R	RNA: RNA	<50	T <sub>R</sub> *; 4×SSC	T <sub>R</sub> *; 4×SSC

- ‡: The hybrid length is that anticipated for the hybridized region(s) of the hybridizing polynucleotides. When hybridizing a polynucleotide to a target polynucleotide of unknown sequence, the hybrid length is assumed to be that of the hybridizing polynucleotide. When polynucleotides of known sequence are hybridized, the hybrid length can be determined by aligning the sequences of the polynucleotides and identifying the region or regions of optimal sequence complementarity.
- $\dagger$ : SSPE (1×SSPE is 0.15M NaCl, 10mM NaH<sub>2</sub>PO<sub>4</sub>, and 1.25mM EDTA, pH7.4) can be substituted for SSC (1×SSC is 0.15M NaCl and 15mM sodium citrate) in the hybridization and wash buffers; washes are performed for 15 minutes after hybridization is complete.
  - ${}^{\star}T_{B}$   $T_{R}$ : The hybridization temperature for hybrids anticipated to be less than

50 base pairs in length should be 5-10°C less than the melting temperature  $(T_m)$  of the hybrid, where  $T_m$  is determined according to the following equations. For hybrids less than 18 base pairs in length,  $T_m(^{\circ}C)=2(\#of\ A+T\ bases)+4(\#of\ G+C\ bases)$ . For hybrids between 18 and 49 base pairs in length,  $T_m(^{\circ}C)=81.5+16.6(\log_{10}[Na^+])+0.41$  (%G+C) - (600/N), where N is the number of bases in the hybrid, and  $[Na^+]$  is the concentration of sodium ions in the hybridization buffer ( $[Na^+]$  for 1×SSC=0.165M).

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Additional examples of stringency conditions for polynucleotide hybridization are provided in Sambrook, J., E.F. Fritsch, and T. Maniatis, 1989, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, chapters 9 and 11, and Current Protocols in Molecular Biology, 1995, F.M. Ausubel et al., eds., John Wiley & Sons, Inc., sections 2.10 and 6.3-6.4, incorporated herein by reference.

Preferably, each such hybridizing polynucleotide has a length that is at least 25% (more preferably at least 50%, and most preferably at least 75%) of the length of the polynucleotide of the present invention to which it hybridizes, and has at least 60% sequence identity (more preferably, at least 75% identity; most preferably at least 90% or 95% identity) with the polynucleotide of the present invention to which it hybridizes, where sequence identity is determined by comparing the sequences of the hybridizing polynucleotides when aligned so as to maximize overlap and identity while minimizing sequence gaps.

122

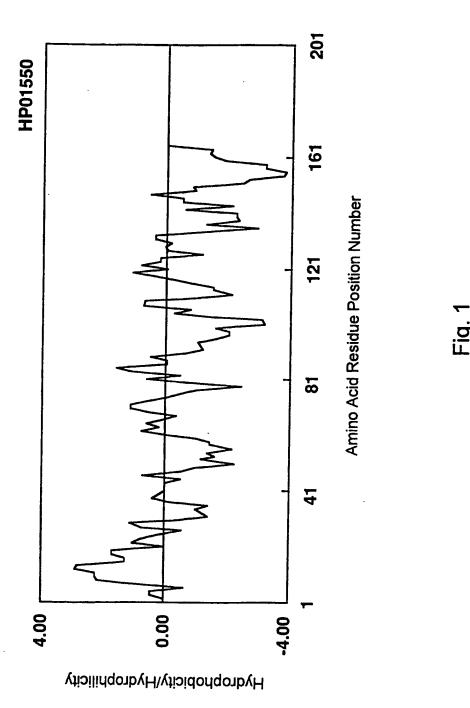
### CLAIMS

1. A protein comprising any one of an amino acid sequence selected from the group consisting of SEQ ID Nos. 1 to 10, 31 to 40, 61 to 70, 91 to 100, and 121 to 130.

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- 2. An isolated DNA coding for the protein according to Claim 1.
- 3. An isolated cDNA comprising any one of a base sequence selected from the group consisting of SEQ ID Nos. 11 to 20, 41 to 50, 71 to 80, 101 to 110, and 131 to 140.
- 4. The cDNA according to Claim 3 consisting of any one of a base sequence selected from the group consisting of SEQ ID Nos. 21 to 30, 51 to 60, 81 to 90, 111 to 120, and 141 to 150.
- 5. An expression vector that is capable of expressing the DNA according to any one of Claim 2 to Claim 4 by in vitro translation or in eucaryotic cells.
  - 6. A transformed eucaryotic cell that is capable of expressing the DNA according to any one of Claim 2 to Claim 4 and of producing the protein according to Claim 1.



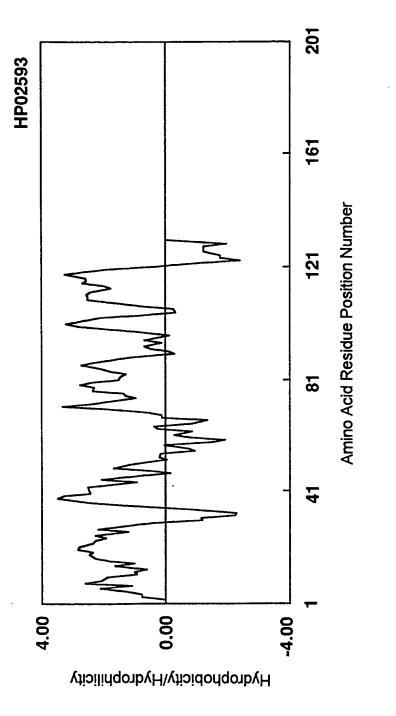


Fig. 2

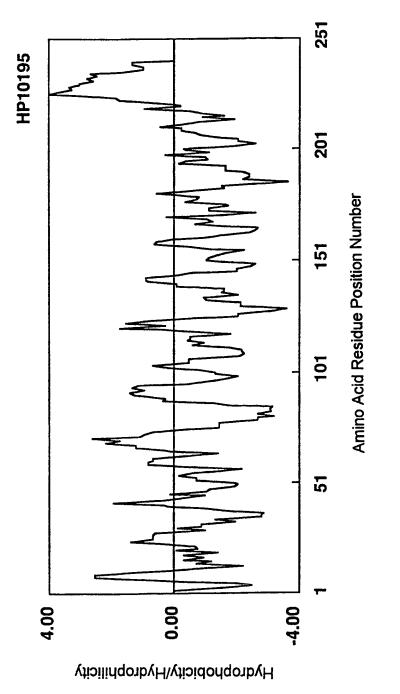


Fig. 3

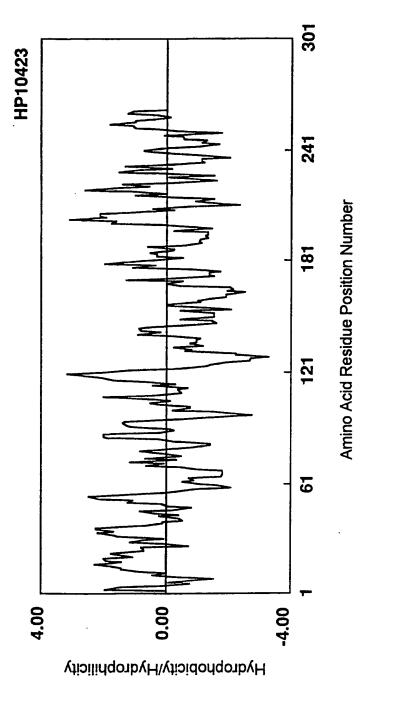
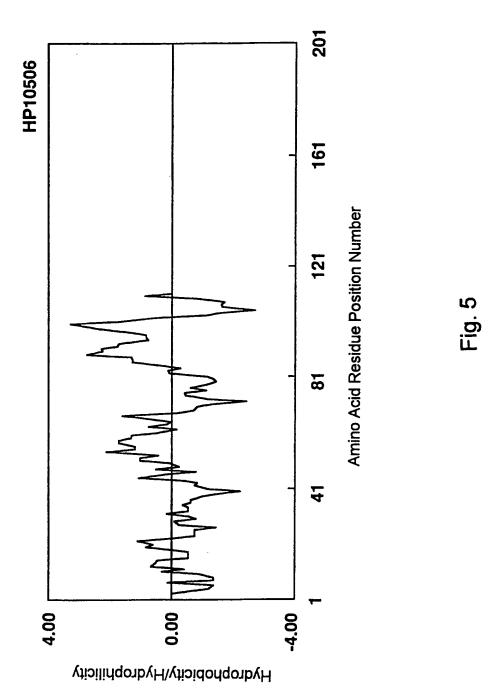
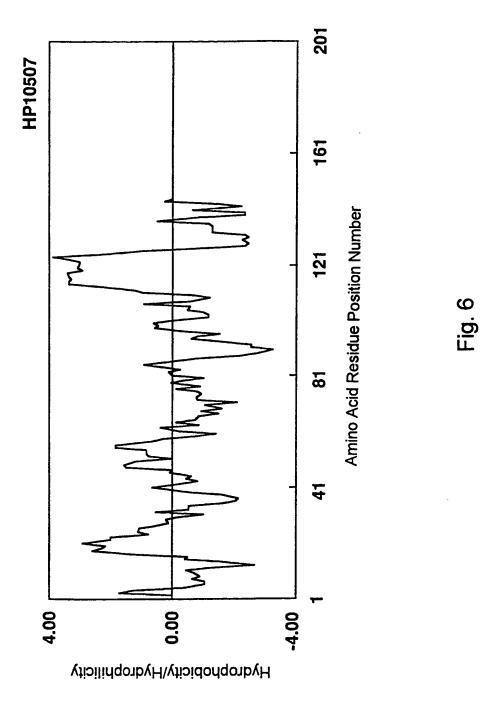


Fig. 4





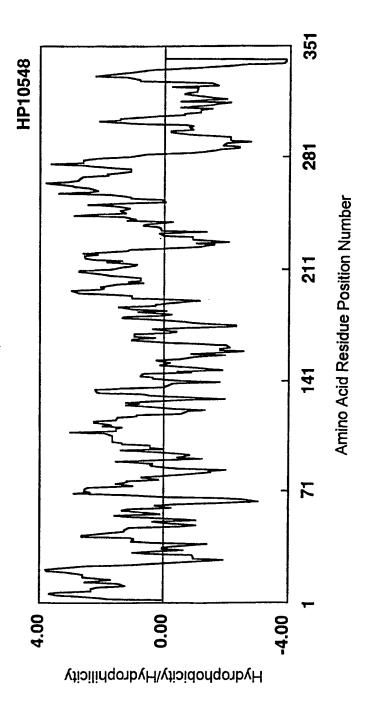


Fig. 7

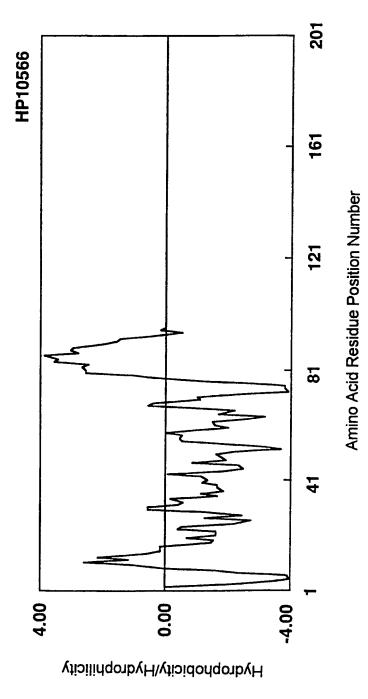
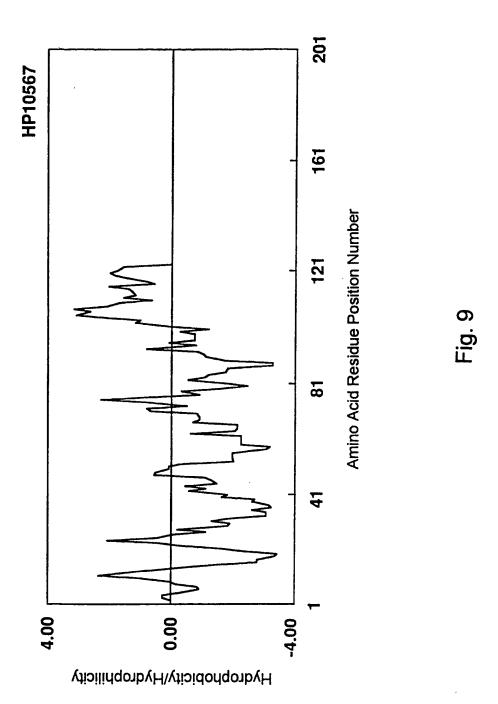


Fig. 8



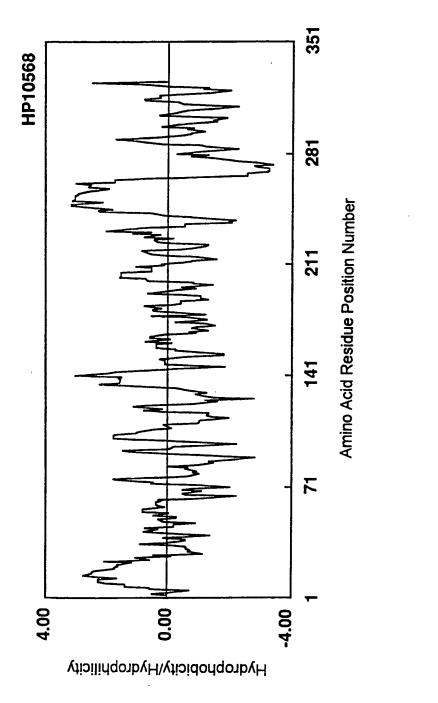


Fig. 10

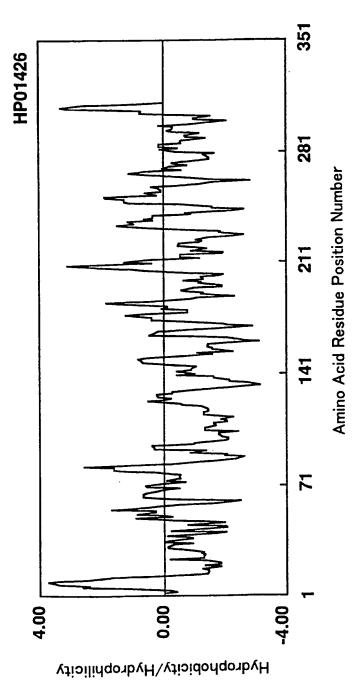


Fig. 11

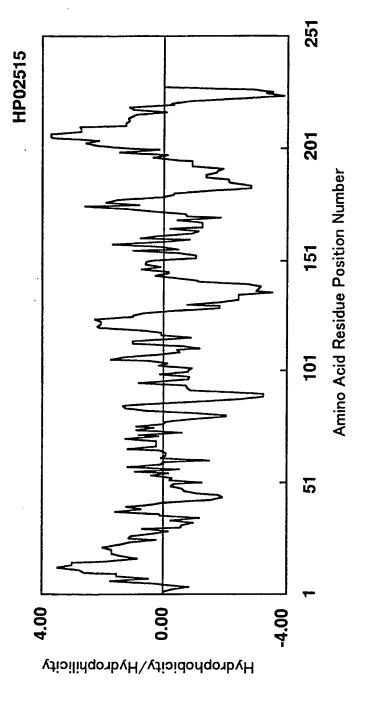
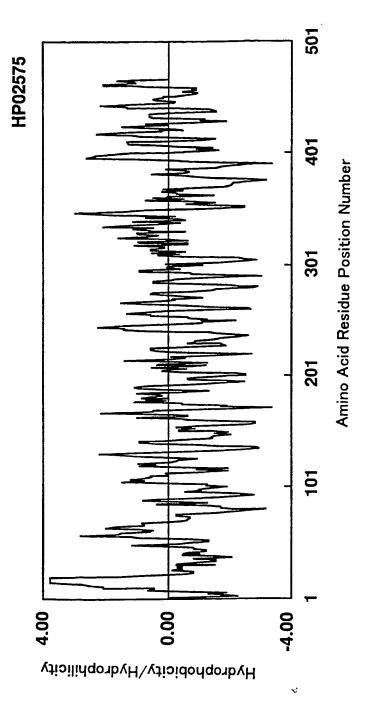


Fig. 12



F18. 13

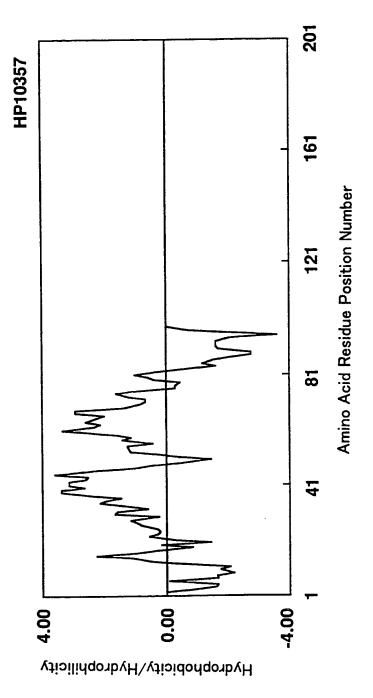


Fig. 14

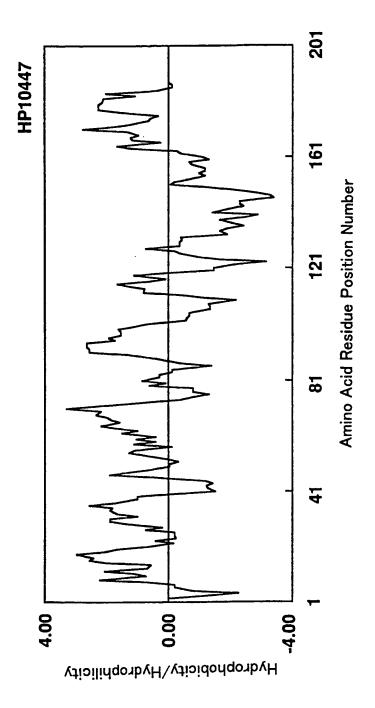


Fig. 15

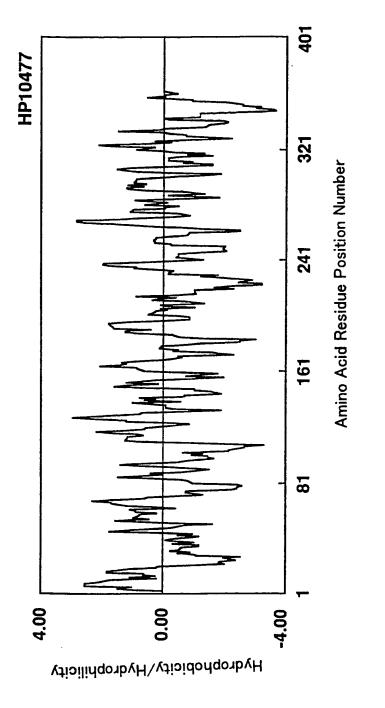


Fig. 16

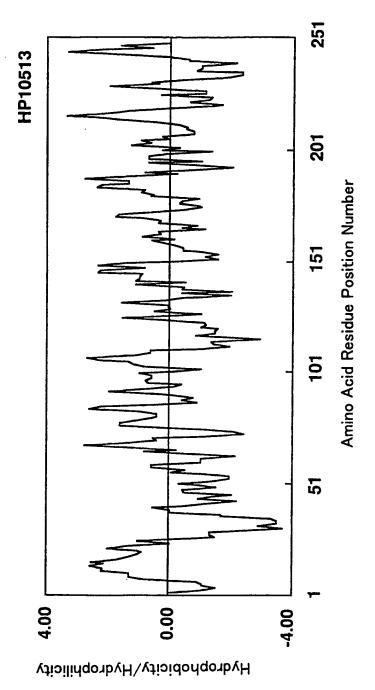


Fig.1 /

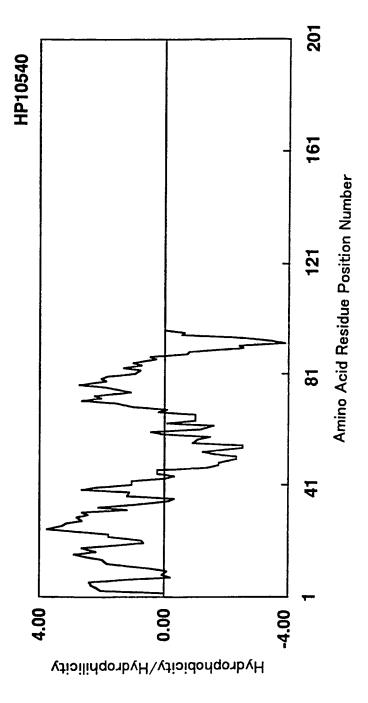


Fig. 18

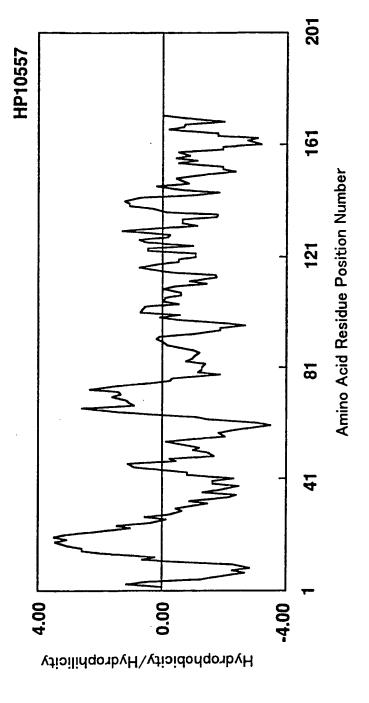


Fig. 19

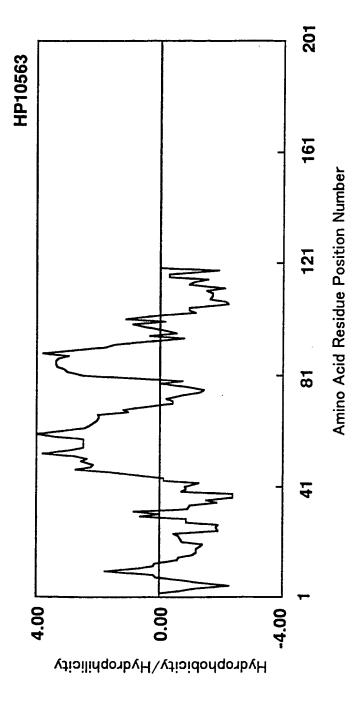


Fig. 20

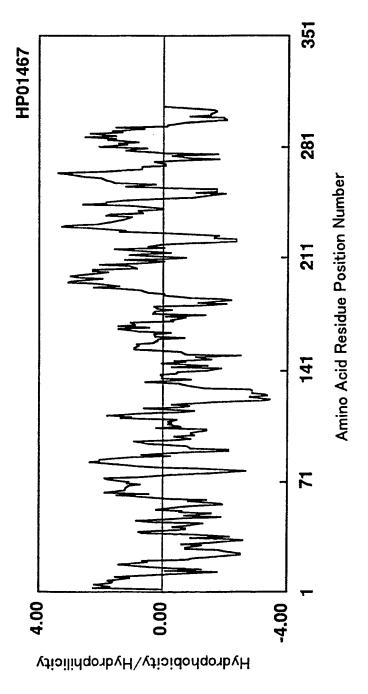


Fig. 21

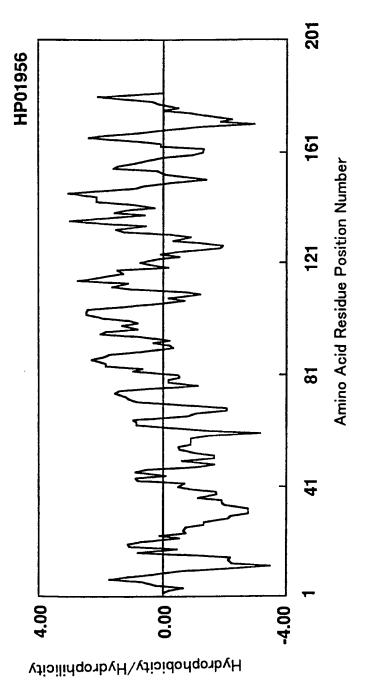


Fig. 22

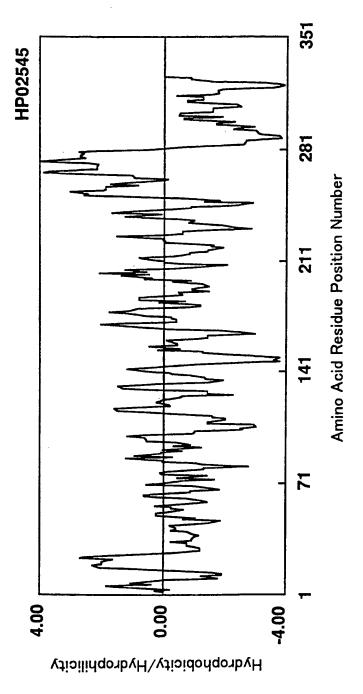


Fig. 23

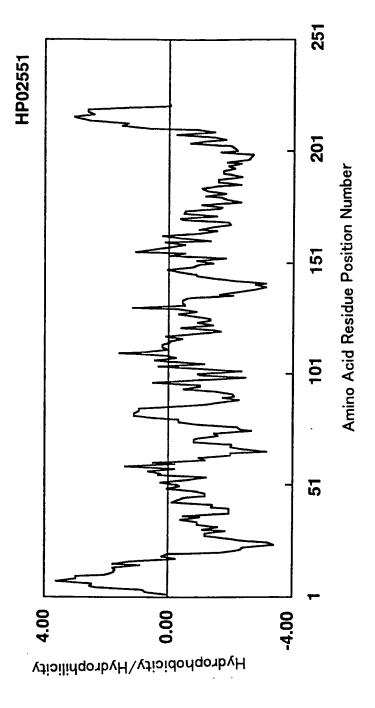


Fig. 24

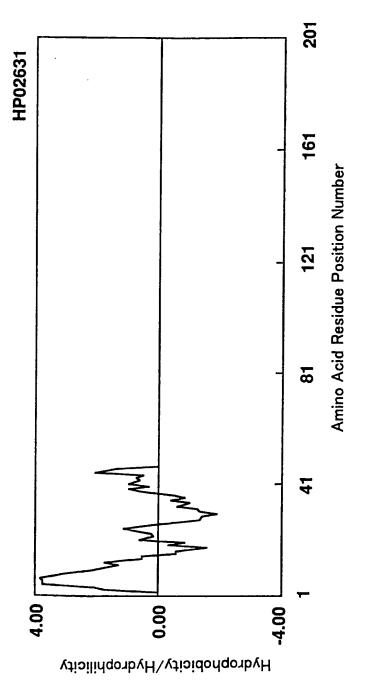


Fig. 25

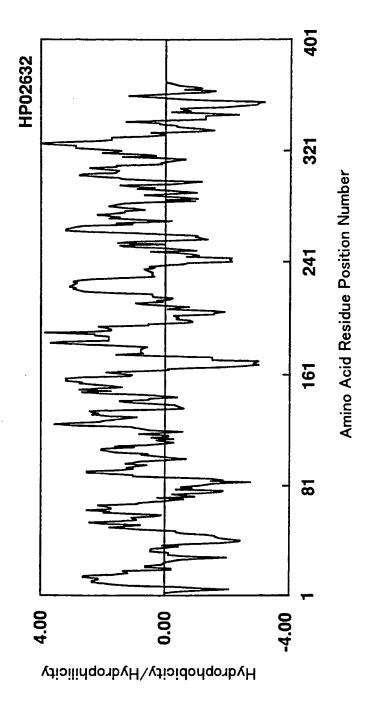


Fig. 26

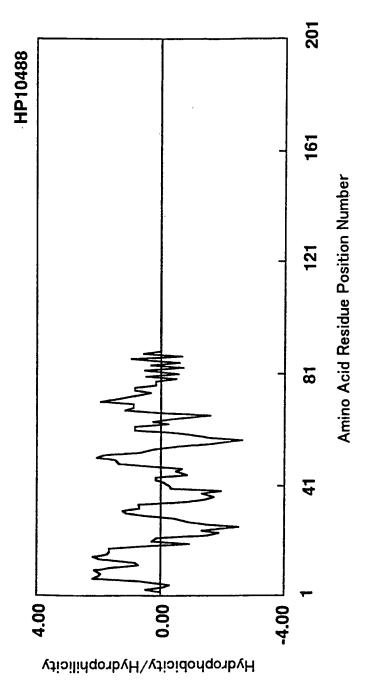


Fig. 27

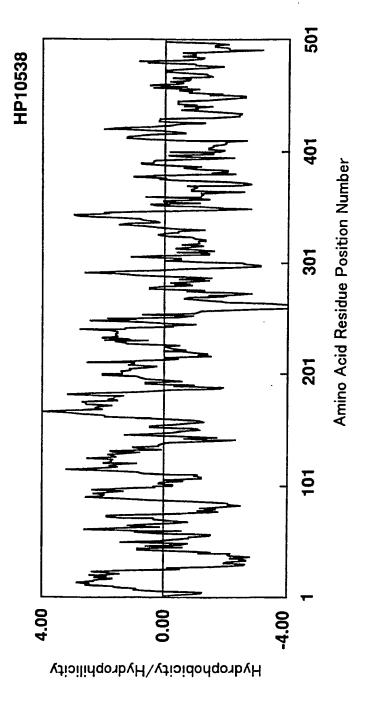


Fig. 28

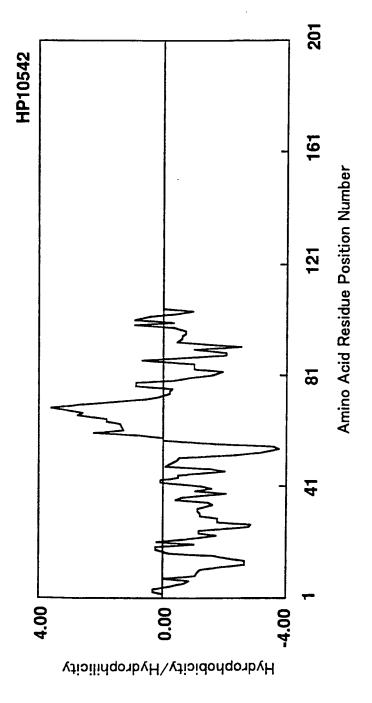


Fig. 29

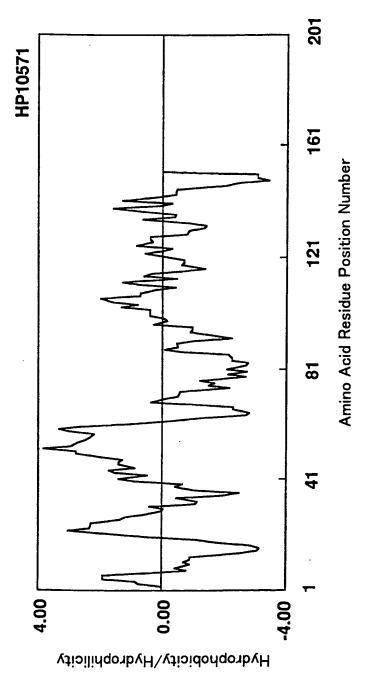


Fig. 30

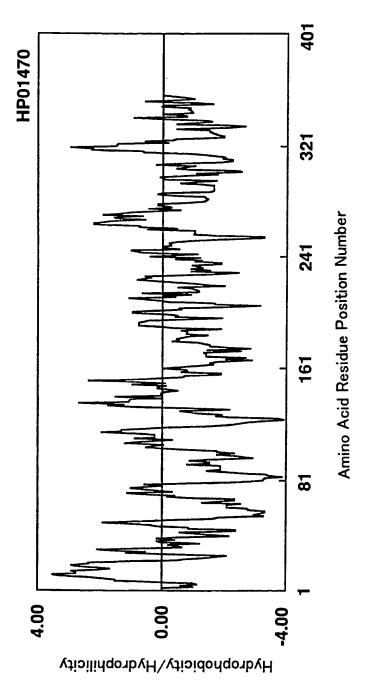


Fig. 31

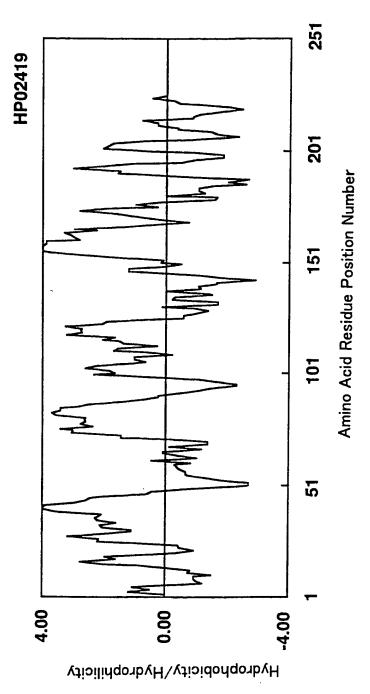


Fig.32

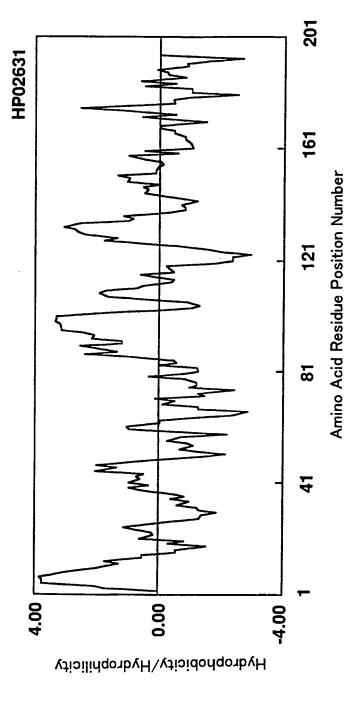


Fig. 33

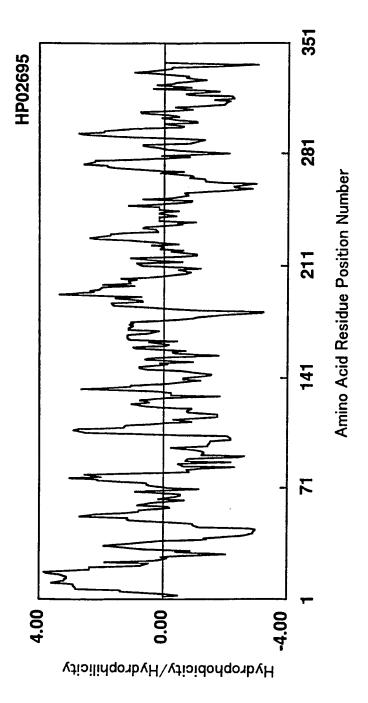


Fig. 34

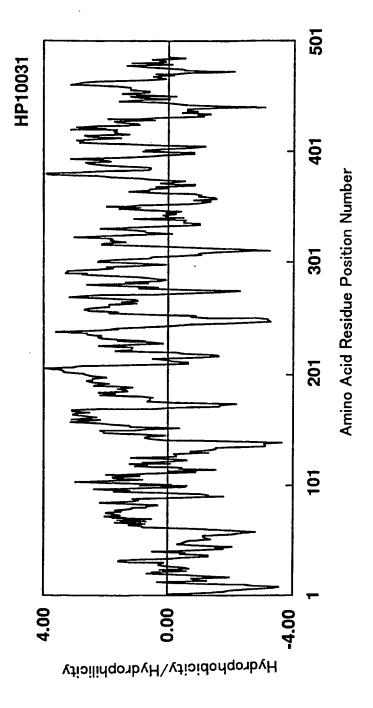


Fig. 35

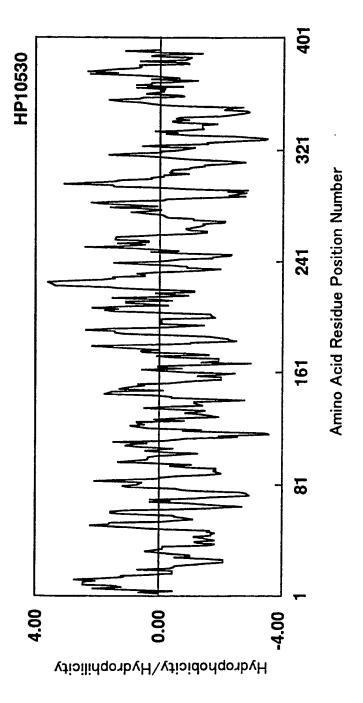


Fig. 36

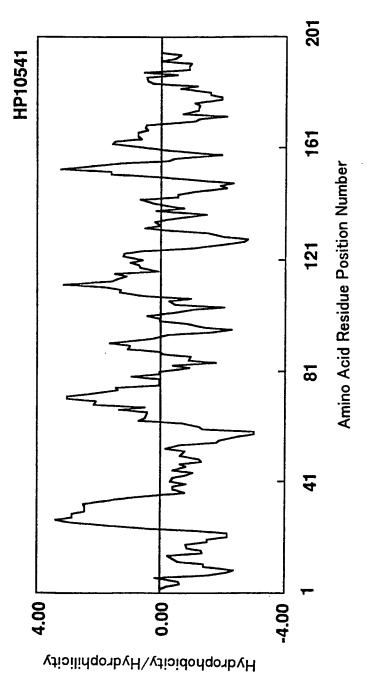


Fig.37

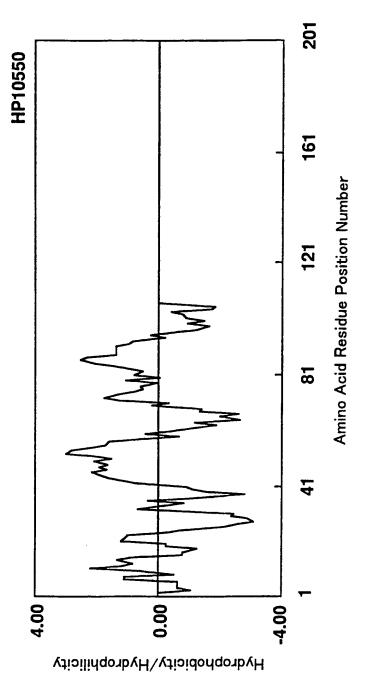


Fig. 38

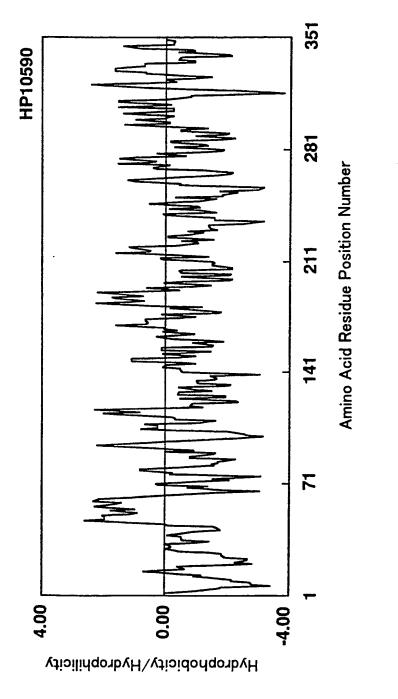


Fig. 39

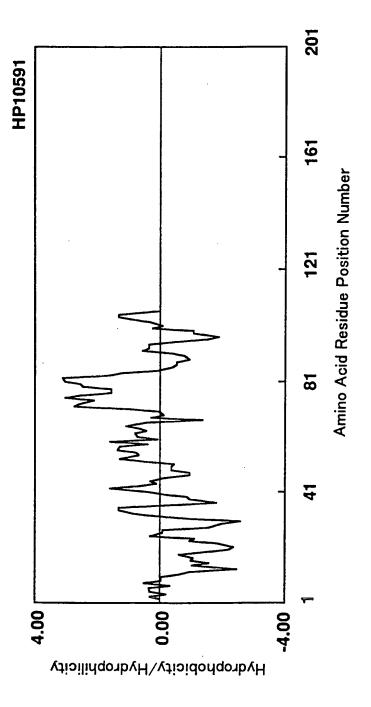


Fig. 40

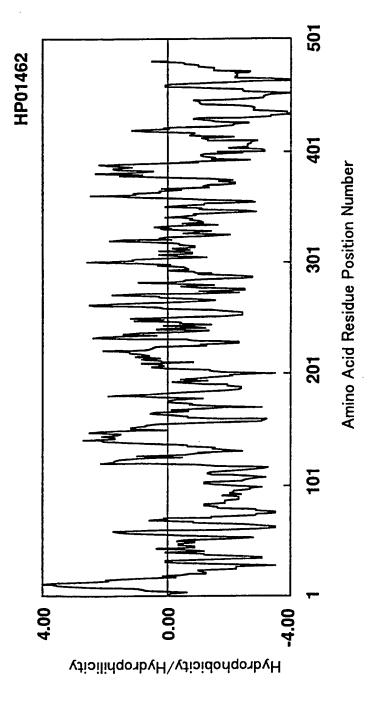


Fig. 41

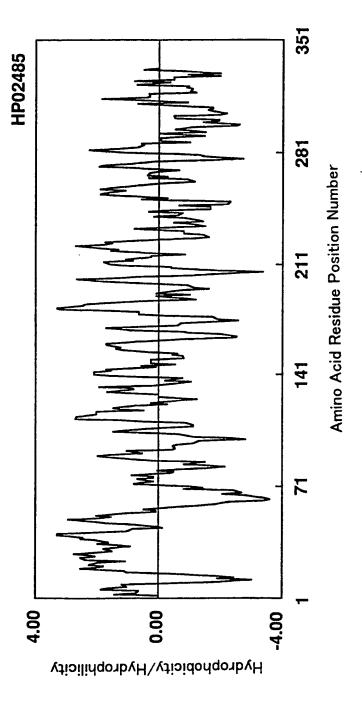


Fig.42

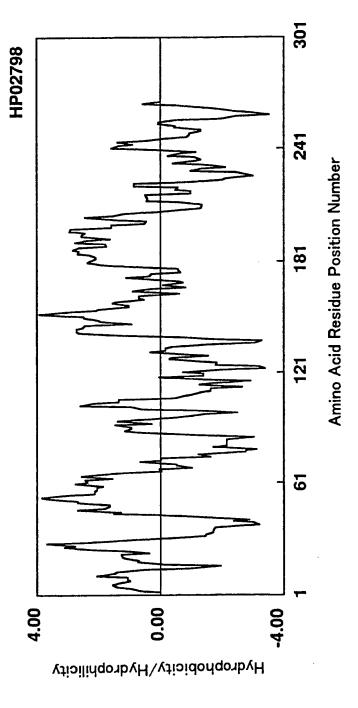


Fig. 43

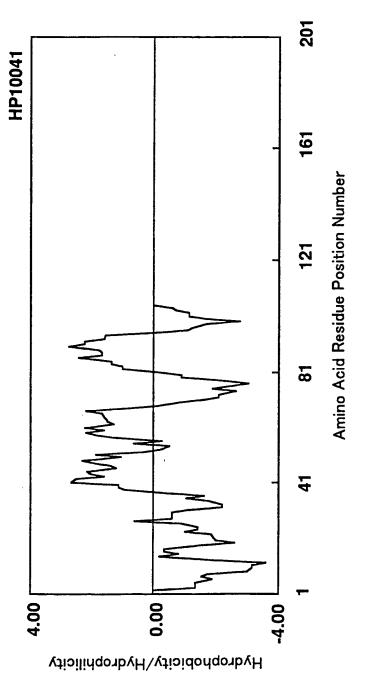


Fig. 44

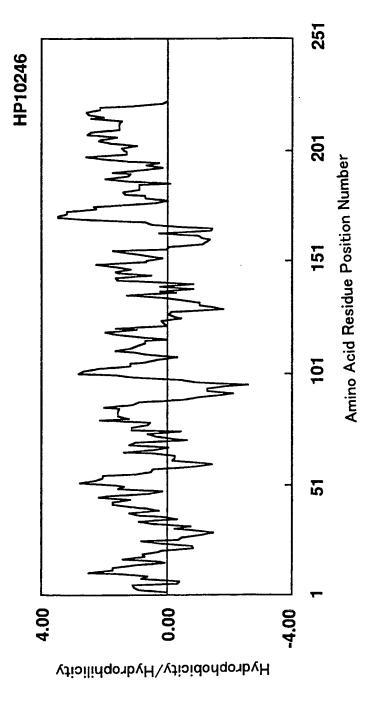


Fig. 45

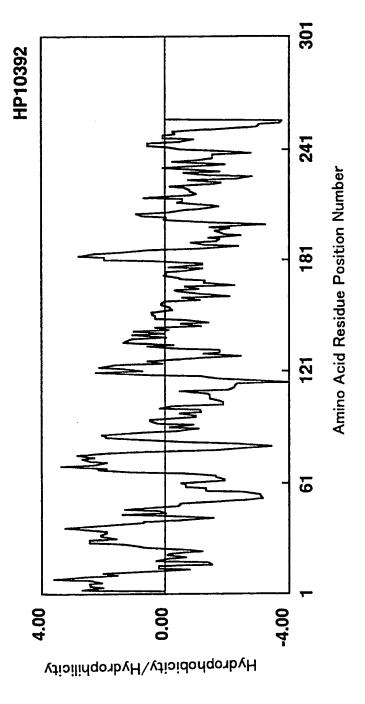


FIg. 46

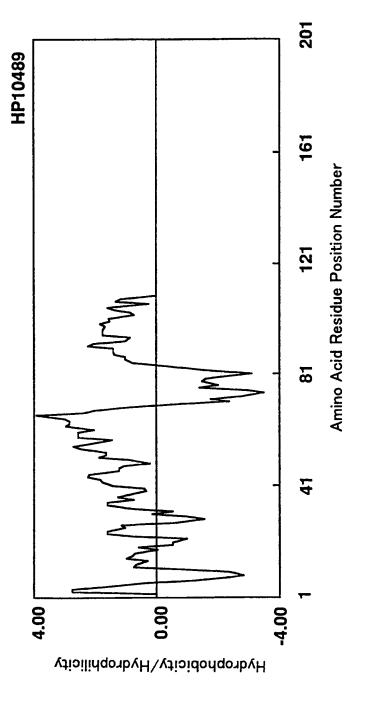


Fig.47

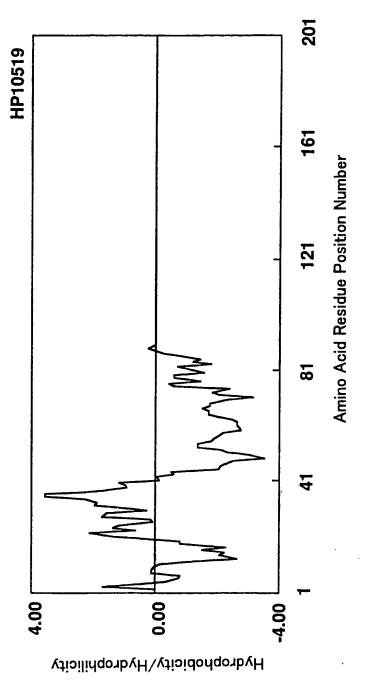


Fig. 48

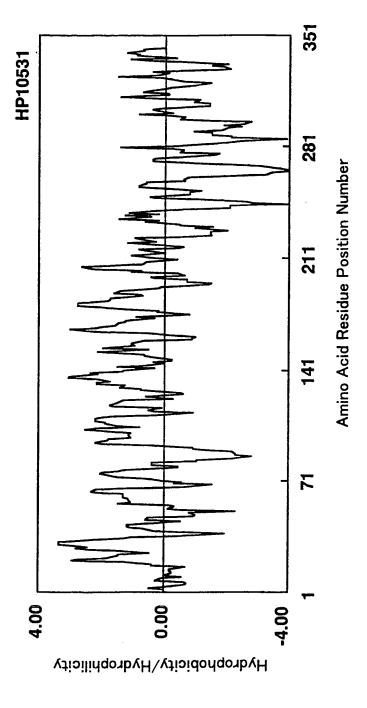


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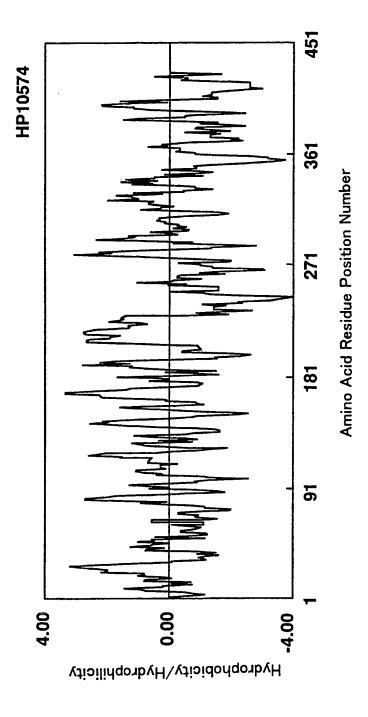


Fig. 50

## 1/177

## Sequence listing

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	ωρ~	) cn	35	Tou	1707	Dwa	Mot	40	C1	A cn	λcn	Pro	45	פות	Mh ∽	mb ~
	THE	Asp 50	Cys	Leu	vai	PIO	55	val	GTÀ	ASII	ASII	60	ıyı	MIG	THE	Thr
	Glu	Gly	Δen	Sor	መከም	Clu		Sar	Tla	Acn	αſΔ		Val.	ጥኒታዮ	Ser	Ton
25	65	GLY	ASII	Jer	1111	70	neu	Der	110	71311	75	O.Lu	741	- 7.		80
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6/177

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	٠٠ د کيل	T.eu	Leu	260	t7⇔1	T C''	m~	Leu	265	בות	πb∽	) en	Gl n	270	መኮ~	N ~~
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8/177

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## 17/177

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	Thr			Met	Gln	Lys		Val	Gln	Asn	Lys		Lys	Ser	Leu	Asn	
10		145					150					155					
10					gaa									_	_		589
			Glu	Met	Glu		Ser	Arg	Cys	Ile		Glu	Ile	Asp	Asp		
	160					165					170					175	
					cgc				-								637
15	GIU	FIIE	cys	TTE	Arg 180	TTE	PIO	СТА	GIY	185	116	THE	гуѕ	Thr	190	Tyr	
10	gat	саа	agg	tat	tet	222	raa.	atc	<b>C22</b>		aca	a++	cta	ata		+++	605
					Ser									_			685
				195		_,_			200		****	<b>,</b>	204	205	275	riie	
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	Leu	Asn	Glu	Trp	Leu	Gln	Ile	Leu	Lys	Pro	Leu	Ser	Asp	Asp	Pro	Thr	
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<b>25</b>	gta	tct	gcc	tca	cgg	tgg	aaa	ata	cca	agt	tct	tgg	aga	tta	ctc	ttt	829
	Val	Ser	Ala	Ser	Arg	Trp	Lys	Ile	Pro	Ser	Ser	Trp	Arg	Leu	Leu	Phe	
	240					245					250					255	
	ggc	agt	ggt	ctt	ccc	cct	gca	ctt	ttc	tgat	ctaa	tt t	ctgt	ttta	t ac	ct	880
	Gly	Ser	Gly	Leu	Pro	Pro .	Ala	Leu	Phe								
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								_	_							tgtat	940
								_				-	-			gtete	1000
			gc t	tttc	atca	t at	gcac	caaa	tgt	aaat	ttt	gtac	aata	aa a	tttt	atttc	1060
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	ggg tet egg ttg tee eag eet ttt gag tee tat ate aet geg eet eee	104
	Gly Ser Arg Leu Ser Gln Pro Phe Glu Ser Tyr Ile Thr Ala Pro Pro	
15	5 10 . 15	
	ggt acc gcc gcg ccc gcc aaa cct gcg ccc cca gct aca ccc gga	152
	Gly Thr Ala Ala Pro Ala Lys Pro Ala Pro Pro Ala Thr Pro Gly	
	20 25 30	
20	geg eeg ace tee eea gea gaa eac ege etg ttg aag ace tge tgg age	200
20	Ala Pro Thr Ser Pro Ala Glu His Arg Leu Leu Lys Thr Cys Trp Ser  35 40 45	
	tgt cgc gtg ctt tct ggg ttg ggg ctg atg ggg gcg ggc ggg tac gtg Cys Arg Val Leu Ser Gly Leu Gly Leu Met Gly Ala Gly Gly Tyr Val	248
	50 55 60 65	
25	tac tgg gtg gca cgg aag ccc atg aag atg gga tac ccc ccg agt cca	296
	Tyr Trp Val Ala Arg Lys Pro Met Lys Met Gly Tyr Pro Pro Ser Pro	250
	70 75 80	
	tgg acc att acg cag atg gtc atc ggc ctc agc att gcc acc tgg ggt	344
	Trp Thr Ile Thr Gln Met Val Ile Gly Leu Ser Ile Ala Thr Trp Gly	
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	atc gtt gtc atg gca gac ccc aaa ggg aag gcc tac cgc gtt gtt t	390
	Ile Val Val Met Ala Asp Pro Lys Gly Lys Ala Tyr Arg Val Val	
	100 105 110	
	gaaagtacca ccagtgaatc tgtettetgt etetgteeet tteecegtga cacacacage	450
35	aggcatggaa tttaatgggt gttctggaca gacacttgta catggacaga catcactact	510

	gtggatacta caagactgag aagaaaatcg tatgttgtca ttctctggct atggagtgtt	57
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	cggtcccgcg cccgaggatc ctccacgggg ctagatggct gcgtcggggg cgggagcgga	120
15	ggtgageggg egetagggee gegageeeee geeggeeett eeteeagege eetgeggaee	180
	cegeagaagg egetegeete eetageeege aaaaacatat egatttttet egetgtggea	240
	acggggacgt cctgatagat cctctgctcc aataggcaac tccggccttc cctgccctga	300
	cctggaacet etgggaggge tgeagagtaa gtgccgccte tgegeteega eggaggcaeg	360
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20	Met Leu	
	1	
	geg ggt gee ggg agg eet gge ete eee eag gge ege eae ete tge tgg	466
	Ala Gly Ala Gly Arg Pro Gly Leu Pro Gln Gly Arg His Leu Cys Trp	
25	5 10 15	
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	Leu Leu Cys Ala Phe Thr Leu Lys Leu Cys Gln Ala Glu Ala Pro Val  20 25 30	
		560
	cag gaa gag aag etg tea gea age ace tea aat ttg eea tge tgg etg	562
30	Gln Glu Glu Lys Leu Ser Ala Ser Thr Ser Asn Leu Pro Cys Trp Leu  35 40 45 50	
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	gtg gaa gag ttt gtg gta gca gaa gag tgc tct cca tgc tct aat ttc	610
	Val Glu Glu Phe Val Val Ala Glu Glu Cys Ser Pro Cys Ser Asn Phe	
	55 60 65	650
35	egg get aaa act ace eet gag tgt ggt eee aca gga tat gta gag aaa	658
50	Arg Ala Lys Thr Thr Pro Glu Cys Gly Pro Thr Gly Tyr Val Glu Lys	

	70	75	80	
	atc aca tgc agc tca tct aag aga	aat gag ttc	aaa agc tgc cgc tca	706
	Ile Thr Cys Ser Ser Ser Lys Arg	Asn Glu Phe	Lys Ser Cys Arg Ser	
	85 90		95	
5	gct ttg atg gaa caa cgc tta ttt	tgg aag ttc	gaa ggg gct gtc gtg	754
	Ala Leu Met Glu Gln Arg Leu Phe	Trp Lys Phe	Glu Gly Ala Val Val	
	100 105		110	
	tgt gtg gcc ctg atc ttc gct tgt	ctt gtc atc	att cgt cag cga caa	802
	Cys Val Ala Leu Ile Phe Ala Cys I	Leu Val Ile	Ile Arg Gln Arg Gln	
10	115 120	125	130	
	ttg gac aga aag gct ctg gaa aag g	gtc cgg aag	caa atc gag tcc ata	850
	Leu Asp Arg Lys Ala Leu Glu Lys V	Val Arg Lys	Gln Ile Glu Ser Ile	
	135	140	145	
	tagetacatt ccaccettgt atcetgggte	ttagagaccc	tatctcagac agtgaaagtg	910
15	aaatggactg atttgcactc ttggttcttt	ggagccttgt	ggtggaatcc ccttttcccc	970
	atcttcttct ttcagatcat taatgagcag	aataaaaaga	gtaaaatggt t	1021
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	ccagggetec agececeagg gaaateteeg	accaggcccg	cccaggagcc agatccaggc	180
30	teetggaaga accatgteeg geagetactg	gtcatgccag	gcacacactg ctgcccaaga	240
	ggagetgetg tttgaattat etgtgaatgt	tgggaagagg	aatgeeagag etgeeggetg	300
	aaaattaccc aaccaagaga aatctgcagg			354
		Met Asp Phe	Leu Val Leu Phe Leu	
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35	tte tae etg get teg gtg etg atg g	gt ctt gtt	ctt atc tgc gtc tgc	402

	Phe	Tyr	Leu	Ala	Ser	Val	Leu	Met	Gly	Leu	Val	Leu	Ile	Cys	. Val	. Cys	
		10					15					20					
	tcg	aaa	acc	cat	agc	ttg	aaa	ggc	ctg	gcc	agg	gga	gga	gca	cag	ata	450
	Ser	Lys	Thr	His	Ser	Leu	Lys	Gly	Leu	Ala	Arg	Gly	Gly	Ala	Gln	Ile	
5	25					30					35					40	
	ttt	tcc	tgt	ata	att	cca	gaa	tgt	ctt	cag	aga	gcc	gtg	cat	gga	ttg	498
	Phe	Ser	Cys	Ile	Ile	Pro	Glu	Cys	Leu	Gln	Arg	Ala	Val	His	Gly	Leu	
					45					50					55		
	ctt	cat	tac	ctt	ttc	cat	acg	aga	aac	cac	acc	ttc	att	gtc	ctg	Cac	546
10	Leu	His	Tyr	Leu	Phe	His	Thr	Arg	Asn	His	Thr	Phe	Ile	Val	Leu	His	
				60					65					70			
	ctg	gtc	ttg	caa	ggg	atg	gtt	tat	act	gag	tac	acc	tgg	gaa	gta	ttt	594
	Leu	Val	Leu	Gln	Gly	Met	Val	Tyr	Thr	Glu	Tyr	Thr	Trp	Glu	Val	Phe	
			75					80					85		,		
15	ggc	tac	tgt	cag	gag	ctg	gag	ttg	tcc	ttg	cat	tac	ctt	ctt	ctg	ccc	642
	Gly	Tyr	Cys	Gln	Glu	Leu	Glu	Leu	Ser	Leu	His	Tyr	Leu	Leu	Leu	Pro	
		90					95					100					
	tat	ctg	ctg	cta	ggt	gta	aac	ctg	ttt	ttt	ttc	acc	ctg	act	tgt	gga	690
	Tyr	Leu	Leu	Leu	Gly	Val	Asn	Leu	Phe	Phe	Phe	Thr	Leu	Thr	Cys	Gly	
20	105					110					115					120	
	acc	aat	cct	ggc	att	ata	aca	aaa	gca	aat	gaa	tta	tta	ttt	ctt	cat	738
	Thr	Asn	Pro	Gly	Ile	Ile	Thr	Lys	Ala	Asn	Glu	Leu	Leu	Phe	Leu	His	
					125					130					135		
	gtt	tat	gaa	ttt	gat	gaa	gtg	atg	ttt	cca	aag	aac	gtg	agg	tgc	tct	786
25	Val	Tyr	Glu		Asp	Glu	Val	Met	Phe	Pro	Lys	Asn	Val	-	Cys	Ser	
				140					145					150			
		_	_					gct	-		_		_	_		=	834
	Thr	Cys		Leu	Arg	Lys	Pro	Ala	Arg	Ser	Lys	His		Ser	Val	Cys	
			155					160					165				
30			_			-		gac			-	-					882
	Asn	_	Cys	Val	His	Arg		Asp	His	His	Cys		Trp	Val	Asn	Asn	
		170					175					180					
				_				agg						•	-		930
		Ile	Gly	Ala	Trp	Asn	Ile	Arg	Tyr	Phe	Leu	Ile	Tyr	Val	Leu	Thr	
35	185					190					195					200	

	ttg	acg	gcc	tcg	gct	gcc	acc	gtc	gcc	att	gtg	agc	acc	act	ttt	ctg	978
	Leu	Thr	Ala	Ser	Ala	Ala	Thr	Val	Ala	Ile	Val	Ser	Thr	Thr	Phe	Leu	
					205					210					215		
	gtc	cac	ttg	gtg	gtg	atg	tca	gat	tta	tac	cag	gag	act	tac	atc	gat	1026
5	Val	His	Leu	Val	Val	Met	Ser	Asp	Leu	Tyr	Gln	Glu	Thr	Tyr	Ile	Asp	
				220			•		225					230			
	gac	ctt	gga	cac	ctc	cat	gtt	atg	gac	acg	gtc	ttt	ctt	att	cag	tac	1074
	Asp	Leu	Gly	His	Leu	His	Val	Met	Asp	Thr	Val	Phe	Leu	Ile	Gln	Tyr	
			235					240					245				
10	ctg	ttc	ctg	act	ttt	cca	cgg	att	gtc	ttc	atg	ctg	ggc	ttt	gtc	gtg	1122
	Leu	Phe	Leu	Thr	Phe	Pro	Arg	Ile	Val	Phe	Met	Leu	Gly	Phe	Val	Val	
		250					255					260					
	GTT	CTG	AGC	TTC	CTC	CTG	GGT	GGC	TAC	CTG	TTG	TTT	GTC	CTG	TAT	CTG	1170
	Val	Leu	Ser	Phe	Leu	Leu	Gly	Gly	Tyr	Leu	Leu	Phe	Val	Leu	Tyr	Leu	
15	265					270					275					280	
	gcg	gcc	acc	aac	cag	act	act	aac	gag	tgg	tac	aga	ggt	gac	tgg	gcc	1218
	Ala	Ala	Thr	Asn	Gln	Thr	Thr	Asn	Glu	Trp	Tyr	Arg	Gly	Asp	Trp	Ala	
					285					290					295		
	tgg	tgc	cag	cgt	tgt	ccc	ctt	gtg	gcc	tgg	cct	ccg	tca	gca	gag	ccc	1266
20	Trp	Cys	Gln	Arg	Cys	Pro	Leu	Val	Ala	Trp	Pro	Pro	Ser	Ala	Glu	Pro	
				300					305					310			
	caa	gtc	cac	cgg	aac	att	cac	tcc	cat	ggg	ctt	cgg	agc	aac	ctt	caa	1314
	Gln	Val	His	Arg	Asn	Ile	His	Ser	His	Gly	Leu	Arg	Ser	Asn	Leu	Gln	
			315					320					325				
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	Glu		Phe	Leu	Pro	Ala	Phe	Pro	Cys	His	Glu	Arg	Lys	Lys	Gln	Glu	
		330					335					340					
				_	tgcc	t tt	gago	tgta	gtt	cccg	ttt	attt	acac	at g	tgga	tcc	1420
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## 27/177

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	Met Thr Lys Lys Lys Arg Glu Asn Leu Gly Val Ala Leu Glu Ile Asp	
	1 - 5 10 15	
	ggg cta gag gag aag ctg tee eag tgt egg aga gae etg gag gee gtg	157
10	Gly Leu Glu Glu Lys Leu Ser Gln Cys Arg Arg Asp Leu Glu Ala Val	
	20 25 30	
	aac tee aga ete cae age egg gag etg age eea gag gee agg agg tee	205
	Asn Ser Arg Leu His Ser Arg Glu Leu Ser Pro Glu Ala Arg Arg Ser	
	35 40 45	
15	ctg gag aag gag aaa aac agc cta atg aac aaa gcc tcc aac tac gag	253
	Leu Glu Lys Glu Lys Asn Ser Leu Met Asn Lys Ala Ser Asn Tyr Glu	
	50 55 60	
	aag gaa ctg aag ttt ctt cgg caa gag aac cgg aag aac atg ctg ctc	301
	Lys Glu Leu Lys Phe Leu Arg Gln Glu Asn Arg Lys Asn Met Leu Leu	
20	65 70 75 80	
	tet gtg gee ate ttt ate ete etg acg ete gte tat gee tac tgg ace	349
	Ser Val Ala Ile Phe Ile Leu Leu Thr Leu Val Tyr Ala Tyr Trp Thr	
	85 90 95	
	atg tgageetgge actteeceae aaccageaea ggetteeaet tggeecet	400
25	Met	
	tgatcaggat caagcaggca cttcaagcct caataggacc aaggtgctgg ggtgttcccc	460
	teccaaceta gtgtteaage atggetteet ggeggeeeag geettgeete eetggeetge	520
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35

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	Met Ala Thr Ser Ser Met Ser Lys Gly Cys Phe	
	1 5 10	•
10	gtt ttt aag cca aac tcc aaa aag aga aag atc tct ctg cca ata gag	158
	Val Phe Lys Pro Asn Ser Lys Lys Arg Lys Ile Ser Leu Pro Ile Glu	
	15 20 25	
	gac tat ttt aac aaa ggg aaa aat gag cct gag gac agt aag ctt cga	206
	Asp Tyr Phe Asn Lys Gly Lys Asn Glu Pro Glu Asp Ser Lys Leu Arg	
15	30 35 40	
	ttc gaa act tat cag ttg ata tgg cag cag atg aaa tct gaa aat gag	254
	Phe Glu Thr Tyr Gln Leu Ile Trp Gln Gln Met Lys Ser Glu Asn Glu	
	45 50 55	•
	cga cta caa gag gaa tta aat aaa aac ttg ttt gac aat ctg att gaa	302
20	Arg Leu Gln Glu Glu Leu Asn Lys Asn Leu Phe Asp Asn Leu Ile Glu	
	60 65 70 75	
	ttt ctg caa aaa tca cat tct gga ttc cag aag aat tca aga gac ttg	350
	Phe Leu Gln Lys Ser His Ser Gly Phe Gln Lys Asn Ser Arg Asp Leu	
	80 85 90	
25	ggc ggt caa ata aaa ctc aga gaa att cca act gct gct ctt gtt ctt	398
	Gly Gly Gln Ile Lys Leu Arg Glu Ile Pro Thr Ala Ala Leu Val Leu	
	95 100 105	
	ggt ata tat gcg tat gtt tgt tca tgc atg cat ctc tgt gta ttt cgt	446
	Gly Ile Tyr Ala Tyr Val Cys Ser Cys Met His Leu Cys Val Phe Arg	
30	110 115 120	
	ttt taaatttttt tttattgttg agaatagtgg aaggacctgt tttgatgage c	500
	Phe	
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35	aaaccaataa aaattootta tottt	585

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																1	
	gcc	gag	ctc	ccg	ggg	ccc	ttt	ctc	tgc	<b>ggg</b>	gcc	ctg	cta	ggc	ttc	ctg	107
15	Ala	Glu	Leu	Pro	Gly	Pro	Phe	Leu	Cys	Gly	Ala	Leu	Leu	Gly	Phe	Leu	
				5					10					15			
							gtg										155
	Cys	Leu		Gly	Leu	Ala	Val	Glu	Val	Lys	Val	Pro		Glu	Pro	Leu	
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	Ser		Pro	Leu	Gly	Lys	Thr	Ala	Glu	Leu	Thr	Cys	Thr	Tyr	Ser	Thr	
		35					40					45					
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o <b>r</b>		Val	Gly	Asp	Ser	Phe	Ala	Leu	Glu	Trp	Ser	Phe	Val	Gln	Pro	Gly	
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	Lys	Pro	Ile	Ser		Ser	His	Pro	Ile		Tyr	Phe	Thr	Asn	_	His	
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no.							aag										347
30	Leu	Tyr	Pro		Gly	Ser	Lys	Ser	_	Arg	Val	Ser	Leu		Gin	Asn	
				85					90					95			
							gee										395
	Pro	Pro		Val	Gly	Val	Ala		Leu	Lys	Leu	Thr	_	Val	His	Pro	
o E	<b>.</b>		100					105					110				
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	Ser	Asp	Thr	Gly	Thi	Tyr	Leu	Cys	Gln	Val	. Asn	Asn	Pro	Pr	o Asj	p Phe	
		115					120	ı				125	;				
	tac	acc	aat	999	ttç	agg	cta	ato	aac	ctt	act	gtg	cto	g gti	t cc	ccc	491
	Tyr	Thr	Asn	Gly	Leu	Gly	Leu	Ile	Asn	Leu	Thr	Val	Let	va.	l Pro	Pro	
5	130					135					140					145	
	agt	aat	ccc	tta	tgo	agt	cag	agt	gga	caa	acc	tct	gto	gga	a ggo	tot	539
	Ser	Asn	Pro	Leu	Cys	Ser	Gln	Ser	Gly	Gln	Thr	Ser	Val	Gl	Gly	/ Ser	
					150					155					160	)	
	act	gca	ctg	aga	tgc	agc	tct	tcc	gag	ggg	gct	cct	aag	CC	gto	, tac	587
10	Thr	Ala	Leu	Arg	Cys	Ser	Ser	Ser	Glu	Gly	Ala	Pro	Lys	Pro	Val	Tyr	
				165					170					175	•		
	aac	tgg	gtg	cgt	ctt	gga	act	ttt	cct	aca	cct	tct	cct	ggo	ago	atg	635
	Asn	Trp	Val	Arg	Leu	Gly	Thr	Phe	Pro	Thr	Pro	Ser	Pro	Gly	Ser	Met	
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15					-			_								ctg	683
	Val		Asp	Glu	Val	Ser	Gly	Gln	Leu	Ile	Leu	Thr	Asn	Leu	Ser	Leu	
		195					200					205					
						tac	-	_		-			_	_		-	731
00		Ser	Ser	Gly	Thr	Tyr	Arg	Cys	Val	Ala		Asn	Gln	Met	Gly	Ser	
20	210					215					220					225	
					_	acc					_					-	779
	Ala	Ser	Cys	Glu		Thr	Leu	Ser	Val		Glu	Pro	Ser	Gln	_	Arg	
					230					235					240		
25				_	_	att				-			_	_	-		827
20	val	ATa	GIY		Leu	Ile	Gly	Val		Leu	Gly	Val	Leu		Leu	Ser	
				245					250					255			
						ctg -										_	875
	Val	AId	260	Pne	Cys	Leu			Pne	GIN	гÀг	GIU		GTÀ	гуѕ	Lys	
30	cca	224						265					270				
00						ggg										_	923
		ப்த 275	GIU	THE	Tyr	Gly		ser	Asp	Leu			Asp	ATa	TTE	Ala	
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	Gln Phe Lys Lys Thr Pro Pro Lys Ile Pro Tyr Lys Ala Ile Ala Leu	
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# 43/177

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#### 46/177

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	cct agt gca ttt gat ggc ctg tat ttt ctc cgc act gag aat ggt gtt	193
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25	ate tac cag ace the tgt gac atg ace tet ggg ggt ggc ggc tgg ace	241
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35	Gly Asp Gly Asn Trp Ala Asn Tyr Asn Thr Phe Gly Ser Ala Glu Ala	363
30	and only use the ure use the use the only set ure did will	

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	Lys	Asp	Leu	Gly	Ile	Trp	His	Val	Pro	Asn	Lys	Ser	Pro	Met	Gln	His	
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	Ser	Gly	Tyr	Gly	Thr	His	Val	Gly	Tyr	Ser	Ser	Ser	Arg	Glu	Ile	Thr	
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#### 51/177

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	Glu	Asp		Ser	Ser	Ser	Phe		Lys	Arg	Gln	Phe		Val	Ser	Lys	
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		Trp	Ser	Asp	Gly	_	GIY	GIÀ	Ala	Pro	_	Gin	Tyr	Trp	Asn		
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10				_	gcc					_	-		-				823
	THE	GTÀ	Pne	ren	Ala 245	тгр	ren	туг	ASN	250	Ser	PIO	vai	Arg		Thr	
	σta	ata	200	+	gat	aat	+~~	<b>~~</b>	aat		200	atc	+ ~+	227	255	er ertr	871
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	Arg	Arg	Glu	Ala	Gly	Ile	Ser	Asp	Tyr	Leu	Thr	Ile	Glu	Glu	Leu	Val	
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	Leu (	Gly	Ala	Thr	Glu	Val	Lys	Leu	Leu	Gly	His	Gly	Gln	Pro	Leu	Asn	
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	tgg a	att	tct	ttg	gag	caa	aat	ggc	att	atg	gta	gaa	ctg	cca	cag	cta	1399
	Trp	Ile	Ser	Leu	Glu	Gln	Asn	Gly	Ile	Met	Val	Glu	Leu	Pro	Gln	Leu	
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	Asn 1	Val	Ile														
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5	Met ·													
	1													
	gat aac gtg cag ccg aaa ata aaa cat cgc ccc ttc tgc ttc agt gtg	164												
	Asp Asn Val Gln Pro Lys Ile Lys His Arg Pro Phe Cys Phe Ser Val													
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	gta tgc tgt ctt gee gae ggg gee ctt att tae egg aag ett etg tte	356												
20	Val Cys Cys Leu Ala Asp Gly Ala Leu Ile Tyr Arg Lys Leu Leu Phe													
	70 75 80													
	aat ccc agc ggt cct tac cag caa aag cct gtg cat gaa aaa aaa gaa	404												
	Asn Pro Ser Gly Pro Tyr Gln Gln Lys Pro Val His Glu Lys Lys Glu													
05	85 90 95													
25	gtt ttg taattttata ttacttttta gtttgatact aagtattaaa	450												
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	catatttetg tattett	467												
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240

292

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Met Glu Glu Gly Asn Leu

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Gly Gly Leu Ile Lys Met Val His Leu Leu Val Leu Ser Gly Ala Trp

10 15 20

1

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Gly Met Gln Met Trp Val Thr Phe Val Ser Gly Phe Leu Leu Phe Arg

25 30 35

age ett eec ega eat ace tte gga eta gtg eag age aaa ete tte eec 436

Ser Leu Pro Arg His Thr Phe Gly Leu Val Gln Ser Lys Leu Phe Pro
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20 ttc tac ttc cac atc tcc atg ggc tgt gcc ttc atc aac ctc tgc atc

484

Phe Tyr Phe His Ile Ser Met Gly Cys Ala Phe Ile Asn Leu Cys Ile

60 65 70

ttg get tea eag eat get tgg get eag ete aca tte tgg gag gee age 532

Leu Ala Ser Gln His Ala Trp Ala Gln Leu Thr Phe Trp Glu Ala Ser
75 80 85

cag ctt tac ctg ctg ttc ctg agc ctt acg ctg gcc act gtc aac gcc 580
Gln Leu Tyr Leu Leu Phe Leu Ser Leu Thr Leu Ala Thr Val Asn Ala

90 95 100

cgc tgg ctg gaa ccc cgc acc aca gct gcc atg tgg gcc ctg caa acc 628

Arg Trp Leu Glu Pro Arg Thr Thr Ala Ala Met Trp Ala Leu Gln Thr

105 110 115

gtg gag aag gag cga ggc ctg ggt ggg gag gta cca ggc agc cac cag

Val Glu Lys Glu Arg Gly Leu Gly Glu Val Pro Gly Ser His Gln

120

125

130

135

ggt ccc gat ccc tac cgc cag ctg cga gag aag gac ccc aag tac agt 724

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	Ala Leu Arg Gln Asn Phe Phe Arg Tyr His Gly Leu Ser Ser Leu Cys	,,,
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Ū	aat ctg ggc tgc gtc ctg age aat ggg ctc tgt ctc gct ggc ctt gcc	820
	Asn Leu Gly Cys Val Leu Ser Asn Gly Leu Cys Leu Ala Gly Leu Ala	021
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	Thr Leu Ala Gly Asn Leu Gly Leu Thr Phe Leu Arg Gly Ser Gln Thr	
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	Gln Ser His Pro Asp Leu Gly Thr Glu Gly Cys Trp Asp Gln Leu Ser	
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	Ala Pro Arg Thr Phe Thr Leu Leu Asp Pro Lys Ala Ser Leu Leu Thr	
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	Phe	Arg	Arg	Gln	Asn	Gly	Ala	Ala	Leu	Thr	Ser	Ala	Ser	Ile	Leu	Ala	
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	His	Leu	Gln	Leu	Gln	Cys	Met	Ser	Gln	Glu	Gln	Leu	Ala	Gln	Val	Ala	
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			235					240					245				
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	Ala	Gly	Leu	Leu	Arg	Pro	Asp	Tyr	Ala	Leu	Leu	Gly	His	Arg	Gln	Leu	
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	Trp	Pro	His	Phe	Thr	Ala	Thr	Val	Lys	Pro	Arg	Pro	Ala	Arg	Ser	Val	
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	Ser	Lys	Arg	Ser	Arg	Arg	Glu	Pro	Pro	Pro	Arg	Thr	Leu	Pro	Ala	Thr	
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	Ser	Ala	Glu	Asp	Leu	Thr	Asp	Gly	Ser	Tyr	Asp	Asp	Val	Leu	Asn	Ala	
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	Val	Ile	Ile	Glu	Arg	Ala	Leu	Ile	Thr	Leu	Gly	Asn	Asn	Ala	Ala	Phe	
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	Ala Trp	Gly	Val	Ile		Leu	Iie	Met	Leu	_	He	Phe	Phe	Asn		
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	Asp	Val	Thr	Ser	Gly	Lys	Glu	Phe	Tyr	Gly	Arg	Gly	Ala	Pro	Tyr	Asn	
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	Asp	Pro	Ala	Asp	Leu	Thr	His	Asp	Thr	Thr	Gly	Leu	Thr	Ala	Lys	Glu	
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	Leu	Glu	Ala	Leu	Asp	Glu	Val	Phe	Thr	Lys	Val	Tyr	Lys	Ala	Lys	Tyr	
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	Pro	Asn	Leu	Asp	Phe	Lys	Pro	Glu	Asp	Gln	Pro	His	Phe	Asp	Ile	Lys	
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#### 63/177

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100

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	Phe	Pro	Thr	Met	Met	Val	Cys	Met	Met	Ala	Trp	Arg	Pro	Ile	Gln	Ala
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#### 67/177

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	Phe	Thr	Ile	Ile	Asn	Ser	Lys	Gln	Met	Gly	Ser	Tyr	Ser	Cys	Phe	Phe
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#### 68/177

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Phe His Phe Gln Thr Gly Gly Arg Asp Ser Cys Thr Met Arg Pro Ser

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Pro Thr Thr Arg Pro Thr Ala Lys Pro Thr Gln Pro Gly Pro Arg Pro 180 185 190

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69/177

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74/177

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## 79/177

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	Tierr	TTE	стА	140	ьеи	met	Gly	Ten	145	Tierr	VIG	val	TYL	150	Cys	GIII	
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#### 91/177

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	Glu Phe Pro Glu Val His Leu Gly Gln Trp Tyr Phe Ile Ala Gly Ala	
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	Ala Pro Thr Lys Glu Glu Leu Ala Thr Phe Asp Pro Val Asp Asn Ile	
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	actggtagca gctggggcta ggagaggctt tetetaggag gcggccgctc gggagcc	357
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	Glu Ala Lys Lys Asn Tyr Tyr Thr Gln Lys Leu His Leu Leu Lys Glu	301
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	Ser Asp Ala Ala Gly Gln Gly Val Ala Ile Thr Gly Asn Gln Thr Phe	
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					-		_		act								933
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	+++	a+a		aat	ata	aac	ccc		gcc	aac	tac	cac		cta	tac	cac	1029
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	Leu Ser Pro Leu Ala Ser Ile Thr Gly Ile Ser Leu Phe Leu Ile Ile	
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#### 99/177

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	Ile Ly	s Gln	Lys	Leu	Glu	Gly	Arg	Pro	Glu	Thr	Glu	Tyr	Arg	Lys	Ala	
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	Gln Th	r Phe	Ser	Gly	His	Glu	Asp	Ala	Leu	Asp	Asp	Phe	Gly	Ile	Tyr	
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10	Glu Ph	e Val	Ala	Phe	Pro	Asp	Val	Ser	Gly	Val	Ser	Arg	Ile	Pro	Ser	
		105					110					115				
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	Arg Se	r Val	Pro	Ala	Ser	Asp	Cys	Val	Ser	Gly	Gln	Asp	Leu	His	Ser	
	12	0				125					130					
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	Thr Va	l Tyr	Glu	Val	Ile	Gln	His	Ile	Pro	Ala	Gln	Gln	Gln	Asp	His	
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	cca ga	g tga	actti	ca t	gggc	taaa	ac ag	rtaca	ttc	gagt	gaaa	ttc	tgaa	agaaa	ac .	600
	Pro Gl	u														
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	tttatt	tcca a	aattt	ctat	c tt	gtta	tttg	tac	aaca	aag	taat	aagg	nat g	gttg	rtcaca	1080
	aaaaca	aaac 1	tatgo	cttc	t ct	tttt	tttc	aat	cacc	agt	agta	tttt	tg a	ıgaag	acttg	1140
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## 100/177

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	Thr	Thr	GIn		Gly	Pro	GIÀ	Arg		GIn	Met	Thr	GIN		Val	Val
	<b>0</b>	•	<b>0</b> 3	180	<b>-</b>	•	••- •	•	185	••-1	<b>.</b>	<b>01</b>	<b>01</b>	190	m\-	
	Cys	Asp		Cys	PIO	ASN	vaı	_	Ten	vaı	Asn	GIU		Arg	Thr	Leu
30	<b>~</b> 1	12n1	195	<b>-</b> 1-	<b>a</b> 1	D	<b>a</b> 1	200	N	3	<b>~</b> 1	Mot	205	M	D==	nh
JU	GIU	Val	GIU	шe	GIU	PIO	-	vaı	Arg	Asp	GTĀ	220	GIU	туг	PIO	Pne
	Tlo	210	<u>را، د</u>	<b>~</b> 1	a1	D=0	215	37 m 7	7	C1v	C1		C1++	3 am	T	<b>3</b>
		Gly	GIU	стА			uts	val	Asp		235	PLO	отЛ	чар		-
	225	Arg	Tla	T 120		230	Tvc	ui ~	Dro			Gl:	Dr.	A ~~		240
35	File	vra	116	_	245	val	nya	นาร		250	r ne	GIU	мц	лгу	G1y 255	изр
<del>5</del> 0					27J					~~0					~~	

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#### 104/177

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	_	•	_		325					330					335	
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	1751	Glu	ו בעז	T +10	85	Lon	T ou	mh.	Lou	-	Pro	Pro	Len	Val.	95	Tou
15	Val	Giu	Vai	100	vsħ	Цец	пеп	TIIL	105	Vul	110	110	Tiệu	110	GIŞ	neu
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30		Ala	ser	Leu	GIĄ	_	Leu	ше	TNE			ше	ren	Ala	Leu	
	225	C	Dh.	<b>n</b>		230	***	T	7 am		235	Mess	7 011	mh ==	D===	240
	PGT	Ser	FIIE		1yr 245	wed	ute	nys	vab	250	ALG	TYL	nen		255	ned
	Val	Cys	ĭ.eu			Ala	Ala	T.eu	ጥኮኮ		Val	Tro	Val			Ala
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#### 109/177

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112/177

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ggt get get tat gag gtt etg tea gat agt gag aaa egg aaa eag tae Gly Ala Ala Tyr Glu Val Leu Ser Asp Ser Glu Lys Arg Lys Gln Tyr 75 80 85 gat act tat ggt gaa gaa gga tta aaa gat ggt cat eag age tee eat		Asp Arg Asn Pro Asp Asp Pro Gln Ala Gln Glu Lys Phe Gln Asp Leu	
Gly Ala Ala Tyr Glu Val Leu Ser Asp Ser Glu Lys Arg Lys Gln Tyr 75 80 85 gat act tat ggt gaa gaa gga tta aaa gat ggt cat cag agc tcc cat	30	55 60 65 70	
75 80 85 gat act tat ggt gaa gaa gga tta aaa gat ggt cat cag agc tcc cat		ggt gct gct tat gag gtt ctg tca gat agt gag aaa cgg aaa cag tac	415
gat act tat ggt gaa gaa gga tta aaa gat ggt cat cag agc tcc cat		Gly Ala Ala Tyr Glu Val Leu Ser Asp Ser Glu Lys Arg Lys Gln Tyr	
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	35	Asp Thr Tyr Gly Glu Glu Gly Leu Lys Asp Gly His Gln Ser Ser His	

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	gga	gac	att	ttt	tca	cac	ttc	ttt	<b>9</b> 99	gat	ttt	ggt	ttc	atg	ttt	gga	511
	Gly	Asp	Ile	Phe	Ser	His	Phe	Phe	Gly	Asp	Phe	Gly	Phe	Met	Phe	Gly	
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	Gly	Thr	Pro	Arg	Gln	Gln	Asp	Arg	Asn	Ile	Pro	Arg	Gly	Ser	Asp	Ile	
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	Ile	Val	Asp	Leu	Glu	Val	Thr	Leu	Glu	Glu	Val	Tyr	Ala	Gly	Asn	Phe	
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	Val	Glu	Val	Val	Arg	Asn	Lys	Pro	Val	Ala	Arg	Gln	Ala	Pro	Gly	Lys	
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					tgt												703
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	Gly	Arg		Gln	Met	Thr	Gln		Val	Val	Cys	Asp		Cys	Pro	Asn	
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	Val		Leu	Val	Asn	Glu		Arg	Thr	Leu	GIu		Glu	He	Glu	Pro	
		200					205					210					
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05		Val	Arg	Asp	Gly		Glu	Tyr	Pro	Phe		GIĄ	GIU	GIÀ	GIu		
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	Thr	TTE		Leu	Val	GIU	ser		vaI	GТĀ	rne	GIU		wab	тте	TNT	
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	His Leu Asp Gly His Lys Val His Ile Ser Arg Asp Lys Ile Thr Arg	
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	Pro Gly Ala Lys Leu Trp Lys Lys Gly Glu Gly Leu Pro Asn Phe Asp	•
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	Leu Lys Gln Gly Ser Val Gln Lys Val Tyr Asn Gly Leu Gln Gly Tyr	
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	ggtgctgccg cctgagtttc aagaattaaa gctgcaagag gactccagga gcaaaagaaa	1530
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																ctc	337
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																ttg	385
	Gly	Val	Trp	Tyr	Leu	Ile			Ala	Val	. Val			Ile	Leu	Leu	
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10	-			-	=											ctg	433
		Ala	Leu	Ala	Asp			Gln	Tyr	Asn			Ser	Ser	Glu	Leu	
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1.5	Gly	Gly	Asp	Phe		Phe	Met	Asp	Asp			Met	Cys	Ile		Ile	
15					65					70					75		
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	Ala	Ile	Ser		Leu	Met	Ile	Leu		Cys	Ala	Met	Ala		Tyr	Gly	
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20	Ala	Tyr	_	Gln	Arg	Ala	Ala	_	Ile	Ile	Pro	Phe			Tyr	Gln	
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	Ser Asp Val Leu Val	Tyr Val Thr Ser Asn Asp	Thr Thr Val Leu Leu	
	190	195	200	
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	Pro Pro Tyr Asp Asp	Ala Thr Val Asn Gly Ala	Ala Lys Glu Pro Pro	
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	Pro Pro Tyr Val Ser	Ala		
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	actgtagttt tcaacatat	g ctttgctgga acactgtgat	agattaactg tagaattctt	1140
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		g ctgcatggga tctggtgccc		1680
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		g aattgggata tatttgatat		1800
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	_	a acattttcag aaaaatgagg		1920
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#### 123/177

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													1				
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	Leu	Leu	Leu	Leu	Val	Ala	Ala	Ser	Ala	Met	Val	Arg	Ser	Glu	Ala	Ser	
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	gcc	aat	ctg	ggc	ggc	gtg	ccc	agc	aag	aga	tta	aag	atg	cag	tac	gcc	150
	Ala	Asn	Leu	Gly	Gly	Val	Pro	Ser	Lys	Arg	Leu	Lys	Met	Gln	Tyr	Ala	
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15	Thr	Gly	Pro	Leu	Leu	Lys	Phe	Gln	Ile	Cys	Val	Ser	Xaa	Gly	Tyr	Arg	
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	cgg	gtg	ttt	gag	gag	tac	atg	cgg	gtt	att	agc	cag	cgg	tac	cca	gac	246
	Arg	Val	Phe	Glu	Glu	Tyr	Met	Arg	Val	Ile	Ser	Gln	Arg	Tyr	Pro	Asp	
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	Ile	Arg	Ile	Glu	Gly	Glu	Asn	Tyr	Leu	Pro	Gln	Pro	Ile	Tyr	Arg	His	
		70					75					80					
	ata	gca	tct	ttc	ctg	tca	gtc	ttc	aaa	cta	gta	tta	ata	ggc	tta	ata	342
	Ile	Ala	Ser	Phe	Leu	Ser	Val	Phe	Lys	Leu	Val	Leu	Ile	Gly	Leu	Ile	
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	att	gtt	ggc	aag	gat	cct	ttt	gct	ttc	ttt	ggc	atg	caa	gct	cct	agc	390
	Ile	Val	Gly	Lys	Asp	Pro	Phe	Ala	Phe	Phe	Gly	Met	Gln	Ala	Pro	Ser	
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	atc	tgg	cag	tgg	ggc	caa	gaa	aat	aag	gtt	tat	gca	tgt	atg	atg	gtt	438
30	Ile	Trp	Gln	Trp	Gly	Gln	Glu	Asn	Lys	Val	Tyr	Ala	Суз	Met	Met	Val	
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	ttc	ttc	ttg	agc	aac	atg	att	gag	aac	cag	tgt	atg	tca	aca	ggt	gca	486
	Phe	Phe	Leu	Ser	Asn	Met	Ile	Glu	Asn	Gln	Суз	Met	Ser	Thr	Gly	Ala	
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35

	Phe Glu Ile Thr Leu Asn Asp Val Pro Val Trp Ser Lys Leu Glu Ser	
	150 155 160	
	ggt cac ctt cca tcc atg caa caa ctt gtt caa att ctt gac aat gaa	582
	Gly His Leu Pro Ser Met Gln Gln Leu Val Gln Ile Leu Asp Asn Glu	
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	atg aag ete aat gtg cat atg gat tea ate eea cae cat ega tea	627
	Met Lys Leu Asn Val His Met Asp Ser Ile Pro His His Arg Ser	
	185 190 195	
	tag caccacctat cagcactgaa aactcttttg cattaaggga tcattgcaag	680
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	cagtgctggc atattttgga attctgcaca ttcatggagt gcaataatac tgtatagett	860
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	tgtgtatgtg cgtgtgatta ccagagaact actaaaaaaa ccaactgctt tttaaatcct	1280
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	Met Asn	
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	Trp	Glu	Leu	Leu	Leu	Trp	Leu	Leu	Val	Leu	Cys	Ala	Leu	Leu	Leu	Leu	
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	ttg	gtg	cag	ctg	ctg	cgc	ttc	ctg	agg	gct	gac	ggc	gac	ctg	acg	cta	214
5	Leu	Val	Gln	Leu	Leu	Arg	Phe	Leu	Arg	Ala	Asp	Gly	Asp	Leu	Thr	Leu	
		20					25					30					
•			-	gag		-		_	-		_			_		_	262
		Trp	Ala	Glu	Trp	Gln	Gly	Arg	Arg	Pro	Glu	Trp	Glu	Leu	Thr	Asp	
	35					40					45					50	
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	Met	Val	Val	Trp		Thr	Gly	Ala	Ser		Gly	Ile	Gly	Glu		Leu	
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	-		_	ttg -					_							_	358
15	Ala	Tyr	Gln	Leu	Ser	Lys	Leu	GTÀ		ser	Leu	Val	Leu		Ala	Arg	
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	Arg	vaı		Glu	Leu	GIU	Arg		гуя	Arg	Arg	cys	95	GIU	ASII	GIÀ	
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20				Glu		-											474
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				Ile													
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	tgc	atg	gat	acc	agc	ttg	gat	gtc	tac	aga	aag	cta	ata	gag	ctt	aac	598
	Cys	Met	Asp	Thr	Ser	Leu	Asp	Val	Tyr	Arg	Lys	Leu	Ile	Glu	Leu	Asn	
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	Tyr	Leu	Gly	Thr	Val	Ser	Leu	Thr	Lys	Cys	Val	Leu	Pro	His	Met	Ile	
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35	Glu	Arg	Lys	Gln	Gly	Lys	Ile	Val	Thr	Val	Asn	Ser	Ile	Leu	Gly	Ile	

### 126/177

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	Arg	Gly	Phe	Phe	Asn	Gly	Leu	Arg	Thr	Glu	Leu	Ala	Thr	Tyr	Pro	Gly	
					215					220					225		
	ata	ata	gtt	tct	aac	att	tgc	cca	gga	cct	gtg	caa	tca	aat	att	gtg	838
	Ile	Ile	Val	Ser	Asn	Ile	Cys	Pro	Gly	Pro	Val	Gln	Ser	Asn	Ile	Val	
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	Glu	Asn	Ser	Leu	Ala	Gly	Glu	Val	Thr	Lys	Thr	Ile	Gly	Asn	Asn	Gly	
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	Phe	Leu	Leu	Val	Thr	Tyr	Leu	Trp	Gln	Tyr	Met	Pro	Thr	Trp	Ala	Trp	
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	Trp	Ile	Thr	Asn	Lys	Met	Gly	Lys	Lys	Arg	Ile	Glu	Asn	Phe	Lys	Ser	
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													aag				1126
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30	Ąsp																
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577

35

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## 146/177

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#### 147/177

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Arg Ile Phe Glu Arg Arg Tyr Gly Ser Arg Lys Phe Ala Ser Phe Leu

35

## 148/177

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## 152/177

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### 153/177

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25	cggagacctc	tctttacagg	aggcctcatc	gggggcctct	tcacctacgt	cctgttctgg	300
	acgttcctct	acggcatggt	gcacgtctac				330
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30	<212> DNA						
	<213> Homo	sapience					
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### 155/177

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	gatgatggaa	`gagggccacc	aggaaaccct	ccccgaagaa	tgggtagaat	caatcatctg	240
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	tttgtgtatg	accttcacgc	agtcaagaac	gacttccaga	tttggaggtt	gatatgtgga	180
	agaataattt	gccttgattt	gaaagatact	ttctgcagta	gtctgcttat	ttataatttt	240
15	aggatatttg	aaagaagata	tggaagcaga	aaatttgcat	cctttttgct	gggttcctgg	300
	gttttgtcag	ccttatttga	ctttctcctc	attgaagcta	tgcagtattt	ctttggcatc	360
	actgcagcta	gtaatttgcc	ttctggattc	ctggcacctg	tgtttgctct	gtttgtacca	420
	ttttactgct	ccataccaag	agtccaagtg	gcacaaattc	tgggtccgtt	gtccatcaca	480
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	atccagagac	agcagagaat	ggagctgctg	gaccggcagc	tgatgttete	tcagtttgca	780
	caagggaggc	gacagagaca	gcagcaggga	ggaatgatca	attggaatcg	tctttttcct	840
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	cccctctag	aagtttctga	ggaacaggtc	gcccggctca	tggagatggg	attttccaga	960
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	geagtgtgga cegegtacet caaegtgtee tggegggtte egeacaeggg agtgaaeegt	180
	acggtgtggg agctgagcga ggagggcgtg tacggccagg actcgccgct ggagcctgtg	240
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	aattteaegg tgeceaeggt ttggggaage aeegtgeaag tetettggtt ggeeeteate	360
	caacgeggeg ggggetgeac ettegeagae aagateeate tggettatga gagagggeg	420
	tetggageeg teatetttaa etteeeeggg accegeaatg aggteateee catgteteae	480
	ccgggtgcag tagacattgt tgcaatcatg atcggcaatc tgaaaggcac aaaaattctg	540
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	cettgggtga ateactatte aattttttte gtttetgtgt cetttttat tattaeggeg	660
	gcaactgtgg gctattttat cttttattct gctcgaaggc tacggaatgc aagagctcaa	720
	agcaggaagc agaggcaatt aaaggcagat gctaaaaaag ctattggaag gcttcaacta	780
	cgcacactga aacaaggaga caaggaaatt ggccctgatg gagatagttg tgctgtgtgc	840
15	attgaattgt ataaaccaaa tgatttggta cgcatcttaa cgtgcaacca tattttccat	900
	aagacatgtg ttgacccatg gctgttagaa cacaggactt gccccatgtg caaatgtgac	960
	atactcaaag ctttgggaat tgaggtggat gttgaagatg gatcagtgtc tttacaagtc	1020
	cctgtatcca atgaaatatc taatagtgcc tcctcccatg aagaggataa tcgcagcgag	1080
	accgcatcat ctggatatgc ttcagtacag ggaacagatg aaccgcctct ggaggaacac	1140
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35	a atg aaa gcc ttc cac act ttc tgt gtt gtc ctt ctg gtg ttt ggg	166

	M	et L	ys A	la P	he H	is T	hr P	he C	ys V	al V	al L	eu L	eu V	al P	he G	ly	
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	agt	gtc	tct	gaa	gcc	aag	ttt	gat	gat	ttt	gag	gat	gag	gag	gac	ata	214
	Ser	Val	Ser	Glu	Ala	Lys	Phe	Asp	Asp	Phe	Glu	Asp	Glu	Glu	Asp	Ile	
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	Val	Glu	Tyr	Asp	Asp	Asn	Asp	Phe	Ala	Glu	Phe	Glu	Asp	Val	Met	Glu	
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	gac	tct	gtt	act	gaa	tct	cct	caa	cgg	gtc	ata	atc	act	gaa	gat	gat	310
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	Glu	Asp	Glu	Thr	Thr	Val	Glu	Leu	Glu	Gly	Gln	Asp	Glu	Asn	Gln	Glu	
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	Gly	Asp	Phe	Glu	Asp	Ala	Asp	Thr	Gln	Glu	Gly	Asp	Thr	Glu	Ser	Glu	
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	Pro	Tyr	Asp	Asp	Glu	Glu	Phe	Glu	Gly	Tyr	Glu	Asp	Lys	Pro	Asp	Thr	
20					100					105					110		
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	Ser	Ser	Ser	_	Asn	Lys	Asp	Pro	Ile	Thr	Ile	Val	Asp		Pro	Ala	
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0.5									tat								550
<b>25</b>	His	Leu		Asn	Ser	Trp	Glu		Tyr	Tyr	Leu	Glu		Leu	Met	Val	
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					-			_	aat								598
	Tnr	-	Leu	Leu	Ala	Tyr		Met	Asn	туг	TIE		GIÀ	ьys	Asn	гÀг	
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30		-	_		-	_	_		ttt								646
		Ser	Arg	Leu	Ala		Ala	Trp	Phe	ASD		HIS	Arg	GIU	ren		
	160					165			<b>4</b> -		170					175	604
		-							gat	-						_	694
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	Pro	Val	Ser	Asp	Gln	Val	Gln	Ile	Lys	Val	Thr	Met	Asn	Asp	Glu	Asp	
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	Met	Asp	Thr	Tyr	Val	Phe	Ala	Val	Gly	Thr	Arg	Lys	Ala	Leu	Val	Arg	
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	Leu	Gln	Lys	Glu	Met	Gln	Asp	Leu	Ser	Glu	Phe	Cys	Ser	Asp	Lys	Pro	
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	Glu	Met	Gly	Glu	Val	Thr	Asp	Gly	Met	Met	Asp	Thr	Lys	Met	Val	His	
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	Leu	Pro	Asp	Thr	Lys	Arg	Thr	Leu	Leu	Phe	Thr	Phe	Asn		Pro	Gly	
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												ctg					1270
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#### 159/177

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	Asn Met Val I	le Tyr Ser Ile	Asp Lys Ala Lys L	ys Phe Arg Leu Asn	
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5	aga gaa ggc a	aa caa aaa gca	gat aag aac cgt g	occ cga gta gaa gag	1366
	Arg Glu Gly I	ys Gln Lys Ala	Asp Lys Asn Arg A	la Arg Val Glu Glu	
	400	405	410	415	
	aac ttc ttg a	aa ctg aca cat	gtg caa aga cag g	aa gca gca cag tct	1414
	Asn Phe Leu L	ys Leu Thr His	Val Gln Arg Gln G	du Ala Ala Gln Ser	
10		420	425	430	
		_		ga atc atg aat gag	1462
	Arg Arg Glu G	lu Lys Lys Arg	Ala Glu Lys Glu A	rg Ile Met Asn Glu	
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	_	-		ct gca ttg agg cgt	1510
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	450		455	460	
				tg aaa caa atc aaa	1558
				et Lys Gln Ile Lys	
20	465	470		75	1610
20		-	cagagattt gagttct	gat gecacetyta	1610
	Val Lys Ala M 480	et			
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				tetgtttgg ggtttggggt	1730
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				aaatctgtg taggttttaa	1850
				acttcagtg tttaaagaaa	1910
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<213> Homo sapience

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	.N	Met Val Glu P	he Ala Pro L	eu Phe Met Pro Trp Glu Arg	
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10	agg ctg cag	g aca ctt gct	gtc cta cag	ttt gtc ttc tcc ttc ttg gca	156
	Arg Leu Gln	n Thr Leu Ala	Val Leu Gln	Phe Val Phe Ser Phe Leu Ala	
	15		20	25	
	ctg gcc gag	g atc tgc act	gtg ggc ttc	ata gee ete etg ttt aca aga	204
	Leu Ala Glu	lle Cys Thr	Val Gly Phe	Ile Ala Leu Leu Phe Thr Arg	
15	30	35		40 45	
	ttc tgg ctc	ctc act gtc	ctg tat gcg	gcc tgg tgg tat ctg gac cga	252
	Phe Trp Leu	Leu Thr Val	Leu Tyr Ala	Ala Trp Trp Tyr Leu Asp Arg	
		50		55 60	
	gac aag cca	cgg cag ggg	ggc cgg cac	atc cag gcc atc agg tgc tgg	300
20	Asp Lys Pro	Arg Gln Gly	Gly Arg His	Ile Gln Ala Ile Arg Cys Trp	
		65	70	75	
	act ata tgg	aag tac atg	aag gac tat	ttc ccc atc tcg ctg gtc aag	348
	_			Phe Pro Ile Ser Leu Val Lys	
0.5	80		85	90	
25				tac att gcg ggc ttc cac ccc	396
		Leu Asp Pro	-	Tyr Ile Ala Gly Phe His Pro	
	95		100	105	
	_			gcc aac ctg tgc act gag agc	444
20			Gly Ala Phe	Ala Asn Leu Cys Thr Glu Ser	
30	110	115	<b></b>	120 125	400
		~		atc cgc ccc cat ctg atg atg	492
	int Gly Phe		Phe Pro Gly	Ile Arg Pro His Leu Met Met	
	nta see the	130	700 0ca ++-	135 140	E 4 0
35	_		_	Pho Arg Agn War Ilo Mot Sor	540
UU	ner tir ren	TTD FUG WLd	TTO LIG	Phe Arg Asp Tyr Ile Met Ser	

				145	•				150					155			
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	Ala	Gly	Leu	Val	Thr	Ser	Glu	Lys	Glu	Ser	Ala	Ala	His	Ile	Leu	Asn	
			160					165					170				
5	agg	aag	ggt	ggc	gga	aac	ttg	ctg	ggc	atc	att	gta	ggg	ggt	gcc	cag	636
	Arg	Lys	Gly	Gly	Gly	Asn	Leu	Leu	Gly	Ile	Ile	Val	Gly	Gly	Ala	Gln	
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	gag	gcc	ctg	gat	gcc	agg	cct	gga	tcc	ttc	acg	ctg	tta	ctg	cgg	aac	684
	Glu	Ala	Leu	Asp	Ala	Arg	Pro	Gly	Ser	Phe	Thr	Leu	Leu	Leu	Arg	Asn	
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	cga	aag	ggc	ttc	gtc	agg	ctc	gcc	ctg	aca	cac	ggg	gca	ccc	ctg	gtg	732
	Arg	Lys	Gly	Phe	Val	Arg	Leu	Ala	Leu	Thr	His	Gly	Ala	Pro	Leu	Val	
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	Met	Gly	Ile	Ser	Leu	Pro	Leu	Phe	His	Gly	Arg	Gly	Val	Phe	Gln	Tyr	
		255					260					265					
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	Ser	Phe	Gly	Leu	Ile	Pro	Tyr	Arg	Arg	Pro	Ile	Thr	Thr	Val	Val	Gly	
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	Lys	Pro	Ile	Glu	Val	Gln	Lys	Thr	Leu	His	Pro	Ser	Glu	Glu	Glu	Val	
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	aac	cag	ctg	cac	cag	cgt	tat	atc	aaa	gag	ctg	tgc	aac	ctc	ttc	gag	1020
30	Asn	Gln	Leu	His	Gln	Arg	Tyr	Ile	Lys	Glu	Leu	Cys	Asn	Leu	Phe	Glu	
				305					310					315			
	gcc	cac	aaa	ctt	aag	ttc	aac	atc	cct	gct	gac	cag	cac	ttg	gag	ttc	1068
	Ala	His	Lys	Leu	Lys	Phe	Asn	Ile	Pro	Ala	Asp	Gln	His	Leu	Glu	Phe	
			320					325					330				
35	tgc	tgaç	jecca	a ag	ggca	gggc	caa	catt	agg	gage	ccag	ca g	gagg	tgct	g		1120

	Cys					
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10	aaaagcctga agcac	aagca ctctccacco	caggcacaca	caccctggaa	ttccctgtgt	1660
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	cacaaagctg accgc	gccat ttcctactca	gcatccttcc	atgaccctcc	attgctccta	1840
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	cattgctggt caagg	ggcac gaacaggtct	ggtgaccctg	caagggagga	gccaggagca	2080
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	caactagaag ctggag	gggaa ggagggcctg	tggctgcagt	ccaggcatgt	aggeeteetg	2200
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## 163/177

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										1			·	5			
				_		-										gga	100
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	Ile	Thr	Leu	Val	Leu	Phe	Leu	His	Asp	Thr	Glu	Leu	Arg	Gln	Trp	Glu	
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		_			_		_							_		ctg	196
15	Glu	Gln	Gly	Glu	Leu	Leu	Leu	Pro	Leu	Thr	Phe	Leu	Leu	Leu	Val	Leu	
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	ggc	tcc	ctg	ctg	ctc	tac	ctc	gct	gtg	tca	ctc	atg	gac	cct	ggc	tac	244
	Gly	Ser	Leu	Leu	Leu	Tyr	Leu	Ala	Val	Ser	Leu	Met	Asp	Pro	Gly	Tyr	
					60					65					70		
20	gtg	aat	gtg	cag	CCC	cag	cct	cag	gag	gag	ctc	aaa	gag	gag	cag	aca	292
	Val	Asn	Val	Gln	Pro	Gln	Pro	Gln	Glu	Glu	Leu	Lys	Glu	Glu	Gln	Thr	
				75					80					85			
	gcc	atg	gtt	cct	cca	gcc	atc	cct	ctt	cgg	cgc	tgc	aga	tac	tgc	ctg	340
	Ala	Met	Val	Pro	Pro	Ala	Ile	Pro	Leu	Arg	Arg	Cys	Arg	Tyr	Cys	Leu	
25			90					95					100				
	gtg	ctg	cag	ccc	ctg	agg	gct	cgg	cac	tgc	cgt	gag	tgc	cgc	cgt	tgc	388
	Val	Leu	Gln	Pro	Leu	Arg	Ala	Arg	His	Cys	Arg	Glu	Cys	Arg	Arg	Cys	
		105					110					115					
	gtc	cgc	cgc	tac	gac	cac	cac	tgc	CCC	tgg	atg	gag	aac	tgt	gtg	gga	436
30	Val	Arg	Arg	Tyr	Asp	His	His	Cys	Pro	Trp	Met	Glu	Asn	Cys	Val	Gly	
	120					125					130					135	
	gag	cgc	aac	cac	cca	ctc	ttt	gtg	gtc	tac	ctg	gcg	ctg	cag	ctg	gtg	484
	Glu	Arg	Asn	His	Pro	Leu	Phe	Val	Val	Tyr	Leu	Ala	Leu	Gln	Leu	Val	
					140					145					150		
35	gtg	ctt	ctg	tgg	ggc	ctg	tac	ctg	gca	tgg	tca	ggc	ctc	cgg	ttc	ttc	532

	Val Leu Leu Trp Gly Leu Tyr Leu Ala Trp Ser Gly Leu Arg Phe Phe	
	155 160 165	
	cag ece tgg ggt etg tgg ttg egg tee age ggg ete etg tte gee aee	580
_	Gln Pro Trp Gly Leu Trp Leu Arg Ser Ser Gly Leu Leu Phe Ala Thr	
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	tte etg etg etg tee ete tte teg ttg gtg g	628
	Phe Leu Leu Ser Leu Phe Ser Leu Val Ala Ser Leu Leu Val	
	185 190 195	
	teg eac etc tac etg gtg gee age aac acc acc tgg gaa ttc atc	676
10	Ser His Leu Tyr Leu Val Ala Ser Asn Thr Thr Thr Trp Glu Phe Ile	
	200 205 210 215	
	tee tea eac ege ate gee tat ete ege eag ege ece age aac eec tte	724
	Ser Ser His Arg Ile Ala Tyr Leu Arg Gln Arg Pro Ser Asn Pro Phe	
_	220 225 230	
15	gac ega ggc etg acc ege aac etg gec eac tte tte tgt gga tgg ecc	772
	Asp Arg Gly Leu Thr Arg Asn Leu Ala His Phe Phe Cys Gly Trp Pro	
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	tca ggg tcc tgg gag acc ctc tgg gct gag gag gaa gag ggc agc	820
	Ser Gly Ser Trp Glu Thr Leu Trp Ala Glu Glu Glu Glu Glu Gly Ser	
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	age cea get gtt tagggttget ggaggeeggg ctacegtett gtgeetga	870
	Ser Pro Ala Val	
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	aaaccacggg gcctgtcccc agctggggtg agcgctcaga gggcctgggg ccctcactcc	930
25	tgcccacgcc teccagaccc cagaacggag ettcaagtca gacagatecc tgccttggtg	990
	ggcagttctg ccttccaagg aagaagggga agaaaaggac ctgtgggtgg ctcaggccca	1050
	agcagacece gggetecace ecageceege ecaggetget gecagtgeac acttttacaa	1110
	atttaatata aagcaagtcc agtctt	1136
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#### 165/177

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	Asn Lys Val Leu Arg Tyr Lys Pro Pro Pro Ser Glu Cys Asn Pro Ala	
	15 20 25	
10	ttg gac gac eeg aeg eeg gac tac atg aac etg etg gge atg ate tte	144
	Leu Asp Asp Pro Thr Pro Asp Tyr Met Asn Leu Leu Gly Met Ile Phe	
	30 35 40	
	age atg tgc ggc etc atg ett aag etg aag tgg tgt get tgg gtc get	192
	Ser Met Cys Gly Leu Met Leu Lys Leu Lys Trp Cys Ala Trp Val Ala	
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	gte tac tgc tec tte ate age ttt gec aac tet egg age teg gag gae	240
	Val Tyr Cys Ser Phe Ile Ser Phe Ala Asn Ser Arg Ser Ser Glu Asp	
	65 70 75	
	acg aag caa atg atg agt agc ttc atg ctg tcc atc tct gcc gtg gtg	288
20	Thr Lys Gln Met Met Ser Ser Phe Met Leu Ser Ile Ser Ala Val Val	
	80 85 90	
	atg tee tat etg cag aat eet cag eee atg aeg eee eea tgg	340
	Met Ser Tyr Leu Gln Asn Pro Gln Pro Met Thr Pro Pro Trp	•
0.5	95 100 105	
25	tgataccage ctagaagggt cacattttgg accetgteta tecactagge etgggetttg	390
	gctgctaaac ctgctgcctt cagctgccat cctggacttc cctgaatgag gccgtctcgg	450
	tgcccccage tggatagagg gaacetggcc ctttcctagg gaacacccta ggcttacccc	510
	teetgeetee etteeeetge etgetgetgg gggagatget gteeatgttt etaggggtat	570
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#### 166/177

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140

135

145

35

	ate gte geg tet get cag gte tgg atg ata aca ege tat gat etg tae	296
	Ile Val Ala Ser Ala Gln Val Trp Met Ile Thr Arg Tyr Asp Leu Tyr	
	150 155 160	
	cac acc ttc cgg cca gct gtc ctc ctg ctg atg ttc ctc agt gtc tac	644
5	His Thr Phe Arg Pro Ala Val Leu Leu Met Phe Leu Ser Val Tyr	
	165 170 175	
	aag gee ttt gtt atg gag ace tte gte cae ete tge teg etg gge agt	692
	Lys Ala Phe Val Met Glu Thr Phe Val His Leu Cys Ser Leu Gly Ser	
	180 185 190	
10	tgg gca gct cta ctg gcc cga gca gtg gta acg ggg ctg ctg gcc ctc	740
	Trp Ala Ala Leu Leu Ala Arg Ala Val Val Thr Gly Leu Leu Ala Leu	
	195 200 205 210	
	age act ttg gee etg tat gte gee gtt gte aat gtg cae tee taggettg	790
	Ser Thr Leu Ala Leu Tyr Val Ala Val Val Asn Val His Ser	
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	gtatttggaa agtt	864
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	Leu Val Tyr Ser Val Pro Arg Leu Ser Arg Trp Leu Ala Gln Pro Tyr	
	10 15 20 25	
	tac ctt ctg tcg gcc ctg ctc tct gct gcc ttc cta ctc gtg agg aaa	147
35	Tyr Leu Leu Ser Ala Leu Leu Ser Ala Ala Phe Leu Leu Val Arg Lys	

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	Leu	Pro	Pro	Leu	Cys	His	Gly	Leu	Pro	Thr	Gln	Arg	Glu	Asp	Gly	Asn	
				45				•	50					55			
5	ccg	tgt	gac	ttt	gac	tgg	aga	gaa	gtg	gag	atc	ctg	atg	ttt	ctc	agt	243
	Pro	Cys	Asp	Phe	Asp	Trp	Arg	Glu	Val	Glu	Ile	Leu	Met	Phe	Leu	Ser	
			60					65					70				
	gcc	att	gtg	atg	atg	aag	aac	cgc	aga	tcc	atg	ttc	ctg	atg	acg	tgc	291
	Ala	Ile	Val	Met	Met	Lys	Asn	Arg	Arg	Ser	Met	Phe	Leu	Met	Thr	Cys	
10		75					80					85					
	aaa	ccc	ccc	cta	tat	atg	ggc	cct	gag	tat	atc	aag	tac	ttc	aat	gat	339
	Lys	Pro	Pro	Leu	Tyr	Met	Gly	Pro	Glu	Tyr	Ile	Lys	Tyr	Phe	Asn	Asp	
	90					95					100					105	
	aaa	acc	att	gat	gag	gaa	cta	gaa	cgg	gac	aag	agg	gtc	act	tgg	att	387
15	Lys	Thr	Ile	Asp	Glu	Glu	Leu	Glu	Arg	Asp	Lys	Arg	Val	Thr	Trp	Ile	
					110					115					120		
	gtg	gag	ttc	ttt	gcc	aat	tgg	tct	aat	gac	tgc	caa	tca	ttt	gcc	cct	435
	Val	Glu	Phe	Phe	Ala	Asn	Trp	Ser	Asn	Asp	Cys	Gln	Ser	Phe	Ala	Pro	
				125					130					135			
20	atc	tat	gct	gac	ctc	tcc	ctt	aaa	tac	aac	tgt	aca	ggg	cta	aat	ttt	483
	Ile	Tyr	Ala	Asp	Leu	Ser	Leu	Lys	Tyr	Asn	Cys	Thr	Gly	Leu	Asn	Phe	
			140					145					150				
•	999	aag	gtg	gat	gtt	gga	cgc	tat	act	gat	gtt	agt	acg	cgg	tac	aaa	531
	Gly	Lys	Val	Asp	Val	Gly	Arg	Tyr	Thr	Asp	Val	Ser	Thr	Arg	Tyr	Lys	
<b>25</b>		155					160					165					
	gtg	agc	aca	tca	ccc	ctc	acc	aag	caa	ctc	cct	acc	ctg	atc	ctg	ttc	579
	Val	Ser	Thr	Ser	Pro	Leu	Thr	Lys	Gln	Leu	Pro	Thr	Leu	Ile	Leu	Phe	
	170					175					180					185	
	caa	ggt	ggc	aag	gag	gca	atg	cgg	cgg	cca	cag	att	gac	aag	aaa	gga	627
30	Gln	Gly	Gly	Lys	Glu	Ala	Met	Arg	Arg	Pro	Gln	Ile	Asp	Lys	Lys	Gly	
					190					195					200		
	cgg	gct	gtc	tca	tgg	acc	ttc	tct	gag	gag	aat	gtg	atc	cga	gaa	ttt	675
	Arg	Ala	Val	Ser	Trp	Thr	Phe	Ser	Glu	Glu	Asn	Val	Ile	Arg	Glu	Phe	
				205					210					215			
35	aac	tta	aat	gag	cta	tac	cag	cgg	gcc	aag	aaa	cta	tca	aag	gct	gga	723

	Asn Leu Asn Glu Leu Tyr Gln Arg Ala Lys Lys Leu Ser Lys Ala Gly	
	220 225 230	
	gae aat ate eet gag gag eag eet gtg get tea ace eee ace aca gtg	771
	Asp Asn Ile Pro Glu Glu Gln Pro Val Ala Ser Thr Pro Thr Thr Val	
5	235 240 245	
	tca gat ggg gaa aac aag aag gat aaa taagateete ac	810
	Ser Asp Gly Glu Asn Lys Lys Asp Lys	
	250 255	
	tttggeagtg etteetetee tgteaattee aggetettte cataaccaca agcetgagge	870
10	tgeageettt tatttatgtt tteeetttgg etgtgaetgg gtgggggage atgeagette	930
	tgattttaaa gaggcatcta gggaattgtc aggcacccta caggaaggcc tgccatgctg	990
	tggccaactg tttcactgga gcaagaaaga gatctcatag gacggagggg gaaatggttt	1050
	ccctccaagc ttgggtcagt gtgttaactg cttatcagct attcagacat ctccatggtt	1110
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	aagtagtgtg teeggegeeg tgtteeaget eegegttgtt eegegagaaa gegagaggee	120
	gageceggge tggtgeg atg gee geg gtg gtg gee aag egg gaa ggg eeg	170
25	Met Ala Ala Val Val Ala Lys Arg Glu Gly Pro	
35	1 5 10	

	ccg	ttc	atc	agc	gag	gcg	gcc	gtg	cgg	ggc	aac	gcc	gcc	gtc	ctg	gat	218
	Pro	Phe	Ile	Ser	Glu	Ala	Ala	Val	Arg	Gly	Asn	Ala	Ala	Val	Leu	Asp	
				15					20					25			
	tat	tgc	cgg	acc	tcg	gtg	tca	gcg	ctg	tcg	ggg	gcc	acg	gcc	ggc	atc	266
5	Tyr	Cys	Arg	Thr	Ser	Val	Ser	Ala	Leu	Ser	Gly	Ala	Thr	Ala	Gly	Ile	
			30					35					40				
	ctc	ggc	ctc	acc	ggc	ctc	tac	ggc	ttc	atc	ttc	tac	ctg	ctc	gcc	tee	314
	Leu	Gly	Leu	Thr	Gly	Leu	Tyr	Gly	Phe	Ile	Phe	Tyr	Leu	Leu	Ala	Ser	
		45					50					55					
10	gtc	ctg	ctc	tcc	ctg	ctc	ctc	att	ctc	aag	gcg	gga	agg	agg	tgg	aac	362
	Val	Leu	Leu	Ser	Leu	Leu	Leu	Ile	Leu	Lys	Ala	Gly	Arg	Arg	Trp	Asn	
	60					65					70					75	
	aaa	tat	ttc	aaa	tca	cgg	aga	cct	ctc	ttt	aca	gga	ggc	ctc	atc	aaa	410
	Lys	Tyr	Phe	Lys	Ser	Arg	Arg	Pro	Leu	Phe	Thr	Gly	Gly	Leu	Ile	Gly	
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	ggc	ctc	ttc	acc	tac	gtc	ctg	ttc	tgg	acg	ttc	ctc	tac	ggc	atg	gtg	458
	Gly	Leu	Phe	Thr	Tyr	Val	Leu	Phe	Trp	Thr	Phe	Leu	Tyr	Gly	Met	Val	
				95					100					105			
	cac	gtc	tac	tgaa	atgg	igg g	lacad	19999	ja ct	tttt	taaa	aaa	ì				500
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			110														
							-									taagt	560
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	ggacaag atg gtt tac atc tcg aac gga caa gtg ttg gac agc cgg agt	109
	Met Val Tyr Ile Ser Asn Gly Gln Val Leu Asp Ser Arg Ser	
	1 5 10	
	cag tot coa tgg aga tta tot ttg ata aca gat tto tto tgg gga ata	157
5	Gln Ser Pro Trp Arg Leu Ser Leu Ile Thr Asp Phe Phe Trp Gly Ile	
	15 20 25 30	
	got gag ttt gtg gtt ttg ttt tto aaa act ctg ctt cag caa gat gtg	205
	Ala Glu Phe Val Val Leu Phe Phe Lys Thr Leu Leu Gln Gln Asp Val	
	35 40 45	
10	aaa aaa aga aga agc tat gga aac tca tct gat tcc aga tat gat gat	253
	Lys Lys Arg Arg Ser Tyr Gly Asn Ser Ser Asp Ser Arg Tyr Asp Asp	
	50 55 60	
	gga aga ggg cca cca gga aac cct ccc cga aga atg ggt aga atc aat	301
	Gly Arg Gly Pro Pro Gly Asn Pro Pro Arg Arg Met Gly Arg Ile Asn	
15	65 70 75	
	cat ctg cgt ggc cct agt ccc cct cca atg gct ggt gga tgaggaaggt	350
	His Leu Arg Gly Pro Ser Pro Pro Pro Met Ala Gly Gly	
	80 85 90	410
20	aaatgtetge tetaagaage agacaacegg acatgegeat teatageaga aggaaaceat	410
20	caagaagtgg aaggetgace atgatgagea gtagatgaat gtgtatgtet aaacaaggac	470
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	cotgactgac atgeagttec ataaatgeag atgtttgtet cattacettt ttgtatagtt	650
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aag age ctt ctg ctg gte cee agt gee ctt ctc ctc ctg cte gee cte  Lys Ser Leu Leu Leu Val Pro Ser Ala Leu Ser Leu Leu Leu Ala Leu 20 25 30  ctc ctg cct cac tge cag aag ctc ttt gtg tat gac ctt cac gca gte Leu Leu Pro His Cys Gln Lys Leu Phe Val Tyr Asp Leu His Ala Val 35 40 45  10 aag aac gac ttc cag att tgg agg ttg ata tgt gga aga ata at	Ser
1	
20   25   30   30   30   30   30   30   30   3	ctc 151
Ctc ctg cct cac tgc cag aag ctc ttt gtg tat gac ctt cac gca gtg CLeu Leu Leu Pro His Cys Gln Lys Leu Phe Val Tyr Asp Leu His Ala Val 35	Leu
Leu Leu Pro His Cys Gln Lys Leu Phe Val Tyr Asp Leu His Ala Val 35 40 45  10 aag aac gac ttc cag att tgg agg ttg ata tgt gga aga ata at	
10	-
10	Val
Lys Asn Asp Phe Gln Ile Trp Arg Leu Ile Cys Gly Arg Ile Ile Cys  50	
S	- ,
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Leu Asp Leu Lys Asp Thr Phe Cys Ser Ser Leu Leu Ile Tyr Asn Phe 15 65 70 70 75 75 80 agg ata ttt gaa aga aga tat gga agc aga aaa ttt gca tcc ttt ttg Arg Ile Phe Glu Arg Arg Tyr Gly Ser Arg Lys Phe Ala Ser Phe Leu 85 90 95 95 ctg ggt tcc tgg gtt ttg tca gcc tta ttt gac ttt ctc ctc att gga 20 Leu Gly Ser Trp Val Leu Ser Ala Leu Phe Asp Phe Leu Leu Ile Glu 100 105 110 110 110 110 110 110 110 110	
15 65 70 75 75 80  agg ata ttt gaa aga aga tat gga agc aga aaa ttt gca tcc ttt ttg Arg Ile Phe Glu Arg Arg Tyr Gly Ser Arg Lys Phe Ala Ser Phe Leu 85 90 95  ctg ggt tcc tgg gtt ttg tca gcc tta ttt gac ttt ctc ctc att gaa  20 Leu Gly Ser Trp Val Leu Ser Ala Leu Phe Asp Phe Leu Leu Ile Glu 100 105 110  gct atg cag tat ttc ttt ggc atc act gca gct agt aat ttg cct tct Ala Met Gln Tyr Phe Phe Gly Ile Thr Ala Ala Ser Asn Leu Pro Ser 115 120 125  25 gga ttc ctg gca cct gtg ttt gct ctg ttt gta cca ttt tac tgc tcc Gly Phe Leu Ala Pro Val Phe Ala Leu Phe Val Pro Phe Tyr Cys Ser 130 135 140  ata cca aga gtc caa gtg gca caa att ctg ggt cg ttg tcc atc aca Ile Pro Arg Val Gln Val Ala Gln Ile Leu Gly Pro Leu Ser Ile Thr 30 145 150 155 160  aac aag aca ttg att tat ata ttg gga ctg cag ctt ttc acc tct ggt Asn Lys Thr Leu Ile Tyr Ile Leu Gly Leu Gln Leu Phe Thr Ser Gly 165 170 175  tcc tac atc tgg att gta gcc ata agt gga ctt atg tcc gtg ctg tcg ttg	
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Arg Ile Phe Glu Arg Arg Tyr Gly Ser Arg Lys Phe Ala Ser Phe Leu 85 90 95 95    ctg ggt tcc tgg gtt ttg tca gcc tta ttt gac ttt ctc ctc att gaa 20    Leu Gly Ser Trp Val Leu Ser Ala Leu Phe Asp Phe Leu Leu Ile Glu 100 105 110    gct atg cag tat ttc ttt ggc atc acc gca gct agt aat ttg cct tct Ala Met Gln Tyr Phe Phe Gly Ile Thr Ala Ala Ser Asn Leu Pro Ser 115 120 125    25 gga ttc ctg gca cct gtg ttt gct ctg ttt gta cca ttt tac tgc tcc Gly Phe Leu Ala Pro Val Phe Ala Leu Phe Val Pro Phe Tyr Cys Ser 130 135	
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100   105   310	_
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Gly Phe Leu Ala Pro Val Phe Ala Leu Phe Val Pro Phe Tyr Cys Ser 130	tcc 487
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Ile Pro Arg Val Gln Val Ala Gln Ile Leu Gly Pro Leu Ser Ile Thr  10 145 150 150 155 160  aac aag aca ttg att tat ata ttg gga ctg cag ctt ttc acc tct ggt  Asn Lys Thr Leu Ile Tyr Ile Leu Gly Leu Gln Leu Phe Thr Ser Gly  165 170 170 175  tcc tac atc tgg att gta gcc ata agt gga ctt atg tcc ggt ctg tgc	aca 535
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	_		ro Asn Asp Leu	ı Val Arg Ile Leu T	
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